

Research and energy independence

DURING the past year, the Nixon Administration has done a rather spectacular U-turn in its public statements on energy research and development. Back in April 1973, when President Nixon was still referring to the looming energy shortages as a challenge rather than a crisis, he said "it is foolish and self-defeating to allocate funds (to energy research and development) more rapidly than they can be effectively spent". But in June he found himself not only promising to spend an extra \$100 million this year, but also announcing that this would be followed by a five-year energy research and development programme which will begin in 1975 and which will eventually soak up \$10,000 million in federal funds. Finally, in November, the President was claiming that this technological effort, coupled with conservation measures, could win independence for the United States from foreign suppliers of oil by 1980.

Welcome as this late espousal of the technological answer may be—particularly for those directly engaged in such research and development—there are nevertheless, strong doubts about whether such a research and development programme will be sufficient to ensure independence. And there are also questions—based as much on the structure of the federal government itself as on the Administration's poor track record in supporting science and technology—about the endurance of the commitment to energy research.

The Administration's research and development effort is likely to follow a set of proposals drawn up by the Chairman of the Atomic Energy Commission at President Nixon's request, and delivered to the White House in December (see *Nature*, **246**, 370; 1973). The proposals, which conformed to President Nixon's suggested ceiling of \$10,000 million for a five-year programme, call for massive concentration on short term goals, such as more effective use of coal and energy conservation, and they also call for almost half the money to be spent on nuclear research and development.

The first thing to be said is that Presidential claims notwithstanding, the suggestion that the United States will be self sufficient in energy by 1980 is nonsense. Even with the \$10,000 million programme, the AEC report suggests that the United States will in fact be importing more oil in 1980 than at present (5.9 million barrels, compared with 5.1 million in 1972). 1985 is the earliest date by which independence can be achieved, the report says, and even that prediction is based on some dubious assumptions.

For one thing, the Administration's plan tacitly assumes that once the federal government has done the research and development and proven the feasibility of energy technologies, private industry will quickly step in and provide the capital necessary to exploit it. Certainly, the AEC's plan calls for some joint industry-government collaboration in setting up pilot plants, but even the Chairman of the Atomic Energy Commission, Dr Dixie Lee Ray, reckoned at a meeting of the President's Energy R&D Advisory Council in December that it will cost as much as \$50,000 million to exploit the fruits of the research and development programme.

A better approach to buying independence for the United States is contained in a bill sponsored chiefly by Senator Henry M. Jackson, which was passed by the Senate at about the same time as the AEC report was sent to the White House. Not only does that bill provide much more money—Jackson reckons as much as \$15,000 million on all energy research and development would be spent between 1975 and 1979 if his bill is adopted—but it also contains an important provision authorising the establishment of joint government-industry development programmes. These would provide federal funds directly for the construction of demonstration plants to exploit new energy technologies and would provide a measure of government control over their operation. That bill is now awaiting action in the House of Representatives, where it demands careful consideration. But even if it survives the Congressional circuit, there is still no guarantee that the money would be spent, for it would then have to go through the appropriations route, and the Administration would also have to be persuaded to spend the money.

100 years ago



IS England rapidly losing that commercial and manufacturing supremacy which she has held before all the world for generations past? Is she going the way of Venice, of Florence, of Holland? If so, is it because she feels blindly secure that "what hath been, will be," neglecting the means on which success in commerce and manufactures in these days depends—means which are being so industriously used by rival nations, that they are rapidly shooting ahead of England on England's own ground?

From *Nature*, 9, 217, January 22, 1873.



international news

Kohoutek makes a good night out

John Hall

Its complex molecules apart, there is no doubt that the biggest let-down of the astronomical year was the arrival of the hapless Dr Kohoutek's comet. After nine months of speculation and counter speculation about the size and brightness of this spectacle it is now possible to make a single dogmatic assertion on the subject without fear of contradiction: that distance lent enchantment to the eye. The thing is not going to bowl over anybody on account of its splendour, and the complex molecules cannot be detected with binoculars.

Turning a blind eye to the dim reality of the phenomenon, at least two British companies had the enterprise to cash in on Kohoutek. Kohoutek Ltd settled for comet 'T' shirts and a million shares at a penny a go, and Transolar Travel plumped for an observation flight, price £12 to £24 a head, which pulled in 140 enthusiasts, culled largely from the ranks of amateur astronomy societies. The media were there to observe the observers and, curiously for an event which was to be enjoyed at a distance of 90 million miles, clutches of spotters milled about with portable tape recorders and instamatic cameras topped with flash cubes which could scarcely have thrown much light on the subject. The British astronomer Mr Patrick Moore was clearly visible to the naked eye.

The tone of the expedition was set at a preliminary lecture, at which a director of Transolar projected an artist's impression of a nineteenth century comet, about as bright as a full moon and stretching half across the sky, which he confessed to be much the sort of show he expected from Kohoutek. This was greeted by polite laughter from the spotters who throughout the lecture insisted on photographing the lecturer, the slides, each other, and such aeroplanes as passed within range.

Once above the Gatwick clouds in a couple of BAC 1-11s, an excited babbling broke out as Venus and Jupiter hove into view, and a gentleman from the BBC paced the gangway soliloquising about the agitation. Then from the flight

deck an announcement was made that, at 33,000 feet, the comet was no longer obscured by cloud, and it was all going to be worthwhile. Patrick Moore raised his voice above all others to issue directions for identifying the target ("left from Venus to Jupiter, then up a bit") and was heavily photographed for his pains.

Then, sure enough, we were able to see a distinct sort of smudge, with a tail extending five degrees maybe, representing perhaps six and a half million miles, and of magnitude three and a half, possibly. Certainly it was a distinct sort of smudge. More plainly, it was possible to make out the street lighting of Wales, Wallasey and the shape of the Mersey estuary, then the Isle of Man, Manchester and, more controversially, Leeds.

The astronomers passed among each other cassette recorders, making essays in historical audio verité. "Have you seen the comet?" "Yes, I've seen the comet. It has a tail extending five de-

grees maybe, representing perhaps six and a half million . . ." and so on. Finally, since there was only one window to every two observers, those who did not have the good fortune to be twisting their necks and spines to crane after the comet, insisted on paying their respects to the captain, since a better view could be had from the flight deck. A visiting rota had to be arranged when the astronomers, firing their flash cubes as they went, were thought to be harassing the pilot. "It's all very well, but we just cannot have people sitting on the captain's shoulders", the Transolar man remonstrated.

Patrick Moore initiated a round of rousing applause for the organisers, who had put on the entire junket with nothing to gain but hard cash, and the only note of concern was voiced when we passed, on the Lancashire coast, a bright flame of petrochemical waste which, I commented, looked remarkably like the exhaust of a SAM-7 missile.

Nuclear safety

Colin Norman, Washington

THE longstanding and bitter dispute about the safety of American light water reactors is about to boil up again in the United States. Next week, the Joint Committee on Atomic Energy is planning to hold three days of public hearings which will put the spotlight on perhaps the most contentious nuclear safety issue: the emergency cooling system which is supposed to flood the reactor core with water if an accident suddenly robs the reactor of coolant. Arguments about whether or not this piece of hardware would actually work in an emergency have been batted back and forth between the Atomic Energy Commission and its critics for the past two or three years. Since the British Government may soon put in an order for American reactors, the matter is of more than parochial interest.

Next week's hearings, which will take place on January 22-24, will be concerned chiefly with a number of regulations issued by the AEC at the end of December. Based on a marathon public inquiry into the functioning of the emergency core cooling system (ECCS), held by the AEC over a four-month period last year, the regulations will reduce the peak operating temperature of light water reactors by 100°F, and put limits

on the amount of oxidation permitted in the fuel cladding.

According to officials of the Atomic Energy Commission, the regulations will reduce the power output from some reactors, and provide an adequate margin of safety in the (unlikely) event of loss of coolant. According to the critics, however, they do no such thing, since the regulations are merely cosmetic changes designed mostly for public relations purposes.

The hearings will also take place against a backdrop of persistent rumours of palace revolts and power struggles among top AEC officials. According to some accounts, open warfare has broken out between Dr Dixie Lee Ray, the Chairman of the AEC, and some of the other commissioners. A number of key officials have resigned from the agency in the past few months, including Mr Milton Shaw, director of the Division of Reactor Technology, Mr Robert E. Hollingsworth, the AEC's General Manager, and Alvin M. Weinberg, Director of the Oak Ridge Laboratory. Moreover, James T. Ramey, a former general counsel of the Joint Committee on Atomic Energy, was reappointed to the Commission last year after his term of office expired. Since Ramey provided a link between the AEC and its Congressional watchdog, his loss has stirred up considerable animosity between Dr Ray and some committee members.

Scientists are playing their part in power saving

John Hall

IN Britain the arrival of the three-day working week, complete with regulations restricting the use of power, raises the spectre of an earlier age of science. Might one expect, without undue romanticism, to be conducting enquiries by the glow of a tallow candle? The speculation, as it happens, turns out to be as inaccurate as it is morbid. By and large, research goes ahead under neon lights, albeit fewer of them, and with power supplies reduced in some cases to 60% of normal and in others not at all.

The Statutory Order restricting the use of electricity offers three channels of escape (at least) which ensure the survival of research programmes whose existence depends on supplies of power. In the first place, work which is taking place at a university is in the clear because of the exemption from cuts allowed to places of education. A further clause in the order allows the operation of apparatus "which has been continuously in use for purposes of research until such time as the operation of the said apparatus can be discontinued without causing loss or damage"—a flexible looking provision, since the order does not actually specify that the loss should be of materials rather than of information. And additionally, the regular supply of power is allowed for the operation of any computer "and any other equipment necessary for the proper functioning thereof". As a last resort a laboratory can make a special plea to regional officers of the Department of Trade and Industry, who have been instructed to avoid "rigid attitudes" in dealing with such applications.

At Jodrell Bank, in spite of a power reduction of 75%, the radio telescopes are still functioning twenty-four hours a day for seven days a week. In fact, says Professor Sir Bernard Lovell, the telescopes use less power than the cooker in the station's canteen. The total load for the entire establishment at present is only 35 kW and even if the regulations were changed in the event of a worsening power situation, Jodrell Bank's own generator could fill the gap for several weeks. The Appleton Laboratory, formerly the Radio and Space Research Station, at Slough is working a five-day week with only three days of power and has had to delay work on certain projects.

University College, London, which hopes to take its electricity for the full five days, but at a voluntarily reduced level of 60% of normal consumption,

also has certain difficulties. As it happens, the Faculty of Medical Sciences has no animal experiments in train and since its other projects were part of teaching programmes, none of its work has been affected. The Department of Chemical Engineering, with a pilot plant which attempts to translate laboratory biochemistry into industrial process design, is, however, a heavy user of power. Large scale fermentation and isolation runs carried out under industrial conditions, can use motor power amounting to 80 horse power.

Dr Peter Dunnill says the department can scarcely afford to have its £0.25 million worth of equipment standing idle but at the same time it is not really feasible to do biological research on a three-day week or at reduced power. Rather, the department will try to take its 60% of power at the normal rate for, say, one full week in every four.

The Royal Aircraft Establishment at Farnborough, reduced to 65% consumption, spread over five days a week, has elected, stoically, to "make do and mend". Computer work and continuous process research is unaffected but testing programmes, including work for Concorde, has to be done "as and when we can manage it", said a spokesman. The nearby National Gas Turbine Establishment is working on the same basis.

The Atomic Energy Authority's research establishments at Culham and Harwell are surviving by using 65% of their November daily average requirement, as are the Dounreay Fast Reactor and the Winfrith Steam Generating Heavy Water Reactor—in spite of the fact that they generate 114 MW between them. Apparently it is considered unethical to plug in your lighting and heating at source.

Naples at the crossroads

THERE is great international interest in the establishment of new conditions at the Naples Zoological Station. The station fulfils for marine biology (along with a number of other stations such as Monaco, Plymouth and Woods Hole) similar functions to those that large accelerators do for nuclear physics. In addition to having a staff of its own, the station is capable of housing large numbers of international visitors. It is not alone among Italian scientific institutions in having recently fallen on hard times as a result of dissatisfaction with the administration among its resident workers.

The Italian government has reacted to discontent within the station by installing a Commissario whilst the administration was overhauled. A new set of statutes has been provided and the financial basis

for the station is much sounder. Italy will provide about £400,000 annually and any country which contributes more than £40,000 annually will be entitled to a seat on the new Administrative Council. It is hoped that Britain will have a seat.

Prospects are much brighter for a settled future but the remaining question is whether the science will be stimulating. This hangs on two issues—who will be the new director and what sort of programmes will he institute? The permanent staff have gone to unusual lengths to ensure that the community is well informed of the needs of the station; a recent advertisement in *Nature* invited suggestions for the development of the institution and by inference nominations for the directorship. It is important at this juncture that, even though the station is understaffed, the decision not be made in a hurry—certainly not until the council has had time to feel its feet.

PSAC reports from the grave

Colin Norman, Washington

THE President's Science Advisory Committee (PSAC) has posthumously complained about the federal government's methods of regulating public exposure to chemicals in the United States. It has also added its ghostly voice to the chorus of opposition to one of the most controversial, and certainly the most effective, pieces of consumer legislation on the lawbooks—the so-called Delaney Amendment which seeks to ban the addition to food of any substance which has been found to cause cancer in animals.

Although PSAC was a victim of President Nixon's post-election massacre of the science advisory apparatus in the United States (the massacre which scrapped the Office of Science and Technology and banished scientists from the White House), its reports continue to surface in Washington as reminders of a past age in science policy making. This latest PSAC report (*Chemicals and Health*, US Government Printing Office, \$2.75), was released last week by Dr H. Guyford Stever, Director of the National Science Foundation, and inheritor of most of the responsibilities which were assigned to the Office of Science and Technology. Stever described the report last week as a "broad, yet intensive, examination of the processes of Federal regulation of chemicals . . . performed in the name of health".

Prepared by a committee which met under the chairmanship of Dr John W. Tukey, Professor of Statistics at Princeton, the report argues for more flexibil-

ity in regulatory decision-making: in other words for risks and benefits to be properly weighed before a chemical is banned or restricted. The committee also argues for more public disclosure of the principles behind regulatory decisions, for public discussion of issues as they arise, and for independent advisory committees to share the burden of regulation with government administrators. Like every other committee which has reported on the matter (and there have been several over the past decade), the PSAC committee also calls for more research into the health effects of environmental chemicals. In particular, it calls for a greater role in environmental research to be assigned to the National Institute of Environmental Health Sciences.

The committee reckons that one of its most important recommendations is that the Administrator of the Food and Drug Administration and the head of the Environmental Protection Agency should each have an advisory board to assist in major regulatory decisions. Such boards would consist of two classes of members: some would be appointed by the administrator from a list suggested by the National Academy of Sciences, and the rest would be drawn from the general public and appointed by the President. The idea would be for the boards to prepare reports and analyses at the administrator's request, and they would also be able to produce reports on their own initiative. Such an arrangement would be analogous in some respects to the Committee on the Safety of Drugs in the United Kingdom, except for the fact that the boards' reports would be made public—a far cry from the obsessive secrecy that surrounds the work of the British committee.

The PSAC report also comes up with the usual recommendations for increasing public participation in, and knowledge of, regulatory issues and decisions. It also makes the obvious—though frequently overlooked—point that the most widespread and damaging effects of exposure to chemicals come from the consumption of alcohol, tobacco and drugs. Programmes designed to protect public health should thus be directed chiefly at reducing consumption of those chemicals, the committee argues.

A more controversial part of the report is concerned with the Delaney Amendment. Although Dr Tukey said last week that the committee does not specifically disapprove of the Delaney Amendment, it is clear that the amendment is not exactly regarded as the epitome of good regulatory policy. What the committee chiefly objects to is the inflexibility that the clause imposes on the administrator of the Food and Drug Agency.

The amendment, which takes its name

from John Delaney, a New York Congressman who is noted for little else, imposes an absolute ban on adding any chemical to food if it has been found to be carcinogenic to animals. The clause has always irked many food manufacturers, because it has led to the banning of chemicals on the basis of what they consider to be unrealistic tests. On the other hand, many cancer scientists have supported the clause because they argue that it should be government policy to reduce public exposure to carcinogens as much as possible. The PSAC committee adopts the position that the Administrator of FDA should have the ability to set safe levels of chemicals in food, on the basis of dose-response studies.

Publication of the report is timely, not only because some of the fiercest and most bitter battles between the federal government and the public continue to take place over the regulation of food additives, drugs and other chemicals in the environment, but also because the energy crisis is forcing the government to backtrack on environmental laws which are designed to protect people against harmful pollutants. Moreover, some trade unions in the United States are taking an increasingly keen interest in the exposure of workers to chemicals in the workplace. The Chemical and Atomic Workers Union, for example, recently held a long strike against the Shell Oil Company over working conditions, in which questions of exposure of workers to carcinogenic chemicals played a large part. Such disputes are clearly going to increase.

Soyuz-13

From our Soviet Correspondent

THE programme of the Soyuz-13 flight (December 18-26, 1973) seems to have been a multipurpose one, combining routine tests of the hardware, astrophysical and geophysical observations, and preparations for possible long term space flights in the future.

The emphasis given to further checks of the on-board systems, to new methods of manual and automatic control, and to autonomic navigation systems, seems at least partly aimed at maintaining the confidence of the United States participants in the forthcoming Soyuz-Apollo linkups. Indeed, one new navigation project involving observations and photographs of luminous effects before sunrise and after sunset, beyond the line of the horizon, can only be relevant for such orbital missions.

The medical checks, which include the new Levkaya apparatus to monitor cerebral circulation during weightlessness, in conditions of rest, and also after graduated exercise with a special expander with a pull of 30 kg at a rate of thirty times a minute, may also be

directed in the first instance towards the joint project, although such data will obviously be of wide application in space medicine.

Similarly, for home consumption (for undercurrents of discontent at the cost of the Soviet space programme still emerge from time to time, mainly in the form of black humour), considerable press coverage has been given to the spectroscopic investigations of earth resources and pollution—now dignified by the name "cosmic ecology". Although this is one of the routine tasks of the Kosmos satellites, it is difficult to see the cost effectiveness of using a manned station for the purpose.

The most interesting feature, however, is that this short term flight has taken over two projects first envisaged for the Salyut station, one astrophysical and one biological. The Orion II telescope (an improved version of the Orion carried by Salyut) was used to carry out extensive ultraviolet spectroscopy of the Sun and stars. The new system includes a wide-field meniscus telescope capable of ultraviolet photography of stars down to magnitude 9.5 or 10.

The other experiment taken over from Salyut is the Oasis enclosed environment recycling system. Oasis originally contained higher plants such as flax and Peking cabbage. The new version, Oasis II, is a microorganism system which consists of two interconnected cylinders. The first cylinder contains bacteria which feed on hydrogen produced by electrolysis, whereas the other contains bacteria which break down urea, absorbing oxygen generated in the first cylinder and liberating carbon dioxide which is then used by the bacteria in the first cylinder. Although only a small scale version has been developed, the Oasis system is considered significant in the development of recycling systems for long duration space flight.

Energy largesse

The Administration's budget, which will be unveiled later this month, will contain some \$1,570 million for energy research and development in the United States, according to Roy L. Ash, Director of the Office of Management and Budget. In an interview with a wire service reporter, Ash said that the Administration has accepted the spending proposals for the 1974-75 financial year contained in the energy research and development plan drawn up last month by the Chairman of the Atomic Energy Commission. The energy research budget next year will thus be some \$700 million above the budget for this year. It will be interesting to see where the money comes from.

news and views

Geomagnetism and climatic change

THERE is a remarkable similarity between the pattern of the Earth's magnetic field over the northern hemisphere, as displayed by the isopleths of magnetic intensity, and the mean height contours of the surfaces of constant atmospheric pressure which define the shape of the circumpolar vortex of (broadly) westerly upper winds which prevail through most of the depth of the troposphere and lower stratosphere. Both patterns have an elongated, dumb-bell-like or bipolar form, at least when averages over a number of years are considered. In both cases, one end of the long axis is in the general region of the Canadian Arctic Archipelago, where the magnetic pole lies, and the other end is over northeast Siberia. In spite of its striking nature, this near-parallelism has received no more than passing notice in the literature of science. In an article on page 131 of this issue of *Nature*, King, of the Appleton Laboratory, Slough directs attention to the matter and adduces a number of other associations between the Earth's magnetic field and the atmospheric circulation and climate. These associations call for serious discussion and investigation, as they may indicate that the parallelism has a physical explanation, possibly of predictive value, in connection with long-term trends in climate.

Among the additional associations claimed by King perhaps the most impressive is just that the distinctive form of the isopleths of constant magnetic intensity over the southern hemisphere, much more nearly circular than in the north but eccentric in relation to the geographical pole with the pattern centred in the Indian Ocean sector, again broadly parallels the prevailing flow of the atmosphere (which has only been established by observation in recent years, roughly since the International Geophysical Year).

Meteorologists have been shy—perhaps understandably shy—about suggestions of a linkage, let alone control (as mentioned by King) of the flow of the atmosphere by the magnetic field, that is, ultimately by the flow in the Earth's molten core. But both phenomena could be registering parallel responses to some other aspect of the physical environment. The reluctance of meteorologists to consider these things doubtless has something to do with the fact that the atmosphere's dynamics and thermodynamics have long been explained adequately for most purposes (but perhaps not for the understanding of all long-term changes affecting climate) in terms of the major items of the energy budget at the Earth's surface and within the atmosphere, together with the effects of friction and distortion of the atmospheric flow by the greatest mountain barriers. Recent years have seen an impressive development of skill in forward calculation of the state of the atmosphere, based on these premises, using the capacity of the giant computers in the service of numerical methods of weather forecasting over one to five days ahead. It is natural that those who have been most closely involved in that particular advance of science should be a little reluctant to admit the need for other considerations

in connection with long-term climatic development and changes—for example, the slow cumulative effects of extraneous influences, terrestrial and extra-terrestrial, and their possible trigger effects—and, perhaps, the appropriateness of quite other methods. It is far from clear, however, that the models used in numerical daily weather forecasting or any climatic models so far in sight will bring any advance at all in the capacity of scientists to forecast the weather a month or more ahead or to meet the demand for prediction of the climatic trend over years and decades ahead; though models can throw light on probable side effects of human activity (for example, increasing the carbon dioxide content of the atmosphere) which may modify the climate of the Earth.

King presents some further riddles in his article, in which a parallelism appears between the long-term displacement of the non-dipole element of the Earth's magnetic field and the behaviour of the atmospheric circulation over the northern hemisphere and its climatic effects suggest parallel histories over recent decades and centuries. Several of these apparent associations may turn out to be of only passing duration, and hence illusory, or they may be no better than loose and qualitative; but the facts will never be known unless they are investigated with the fullest assembly of long-term data that can be marshalled to the task. There is a clear, and urgent, need to identify and understand the physical processes that are involved in climatic changes—a need stressed, even before the current energy crisis, by the upsets to the international economy during the past 5 to 15 years by the long-continued, and increasing, drought in parts of Africa and several individual years of harvest failure over wide areas in India, China and the Soviet grainlands in central Asia.

Among the variables that have to be brought into the consideration of how climate varies, solar disturbance and minute, but directed, inputs of energy in high geomagnetic latitudes are often suggested. The ionosphere is well known to be affected by solar activity. Any responses in the lower atmosphere or, through the agency of geomagnetic disturbances, in the Earth's molten core are likely to differ with the time-scale of the external trigger operation; but they may show some common features and they are perhaps both likely to be more important in the case of long-lasting changes, however small in origin. One noticeable difference may already have been observed, which should warn meteorologists against expecting too close, or too complete, a parallelism: there is no suggestion so far that the geomagnetic field has registered a three-pole field over the northern hemisphere like that which has often appeared in the northern hemisphere atmosphere during the past 13 years (the third pole being near Novaya Zemlya.) Surely, however, no such strong hints of association as King points to should be left uninvestigated, especially when better knowledge of the processes of climate and climatic change is urgently needed. Another possibility is that such investigations may throw some light on the probable evolution of the flow in the Earth's molten core.

H. H. L.

Search for catecholamine receptors

CONSIDERABLE progress has been made recently in the identification of membrane-bound receptor structures for polypeptide hormones such as insulin, glucagon and corticotrophin in mammalian tissues and cholinergic receptors in the electric organs of fish and eels. These studies have been based on the use of radioactive hormones or antagonists which bind very selectively to the receptor sites. Such binding if it is to be a valid method for identifying the receptors should show a very strict specificity, it should be saturable and indicate a finite and usually small number of receptor sites, and the affinity and rate constants for the binding reaction should correlate with the known biological properties of the receptor stimulant or antagonist molecule used. These criteria have been fulfilled in the instances just cited, and similar approaches have been successfully used to identify cholinergic muscarinic binding sites in mammalian brain, glycine receptor sites in spinal cord and receptors for morphine and other opiate drugs in brain (Hiley *et al.*, *Biochem. J.*, **127**, 86P; 1972; Young and Snyder, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2832; 1973; Pert and Snyder, *Science, N.Y.*, **179**, 1011; 1973).

Cuatrecasas and colleagues, however, in last week's issue of *Nature*, (247, 92; 1974), illustrate the many pitfalls that await the unwary in using this apparently simple experimental approach for identifying receptor structures. Tritium-labelled noradrenaline of high specific activity has been available for some years, and might seem to offer a simple approach for labelling and identifying catecholamine receptors in mammalian tissues. Indeed Lefkowitz and his colleagues (*Proc. natn. Acad. Sci. U.S.A.*, **68**, 1773; 1971; *J. biol. Chem.*, **248**, 342; 1973) have claimed that the binding of ^3H -noradrenaline to fragments of cell membrane in microsomal fractions from mammalian heart and liver represents a specific binding to β adrenoreceptors in these tissues. Cuatrecasas *et al.* have carefully assessed this claim and their article clearly refutes it.

The binding of labelled noradrenaline to membrane fragments from liver, heart and fat cells or to intact fat cells was saturable, but the number of binding sites present in fat cells was three to four orders of magnitude greater than the number of insulin or glucagon receptor sites measured pre-

viously in these cells. Furthermore, the binding of ^3H -noradrenaline did not obey the strict specificity that would be predicted if this binding did represent labelling of β adrenoreceptors. For example, the non-radioactive (+) and (-) stereoisomers of noradrenaline had identical affinities as competitive antagonists of ^3H -noradrenaline binding. The affinity constant for such inhibition was more than $1\mu\text{M}$, whereas the apparent affinity constant of (-) noradrenaline at β adrenoreceptors in fat cells was less than $0.1\mu\text{M}$, and (+) noradrenaline was at least one thousand times less potent in eliciting β -adrenoreceptor responses. ^3H -noradrenaline binding was also antagonised quite effectively by various catechol compounds, including pyrogallol and catechol acids, none of which have any activity at all as β -adrenoreceptor stimulants, nor do they antagonise the receptor actions of (-) noradrenaline or isoprenaline. On the other hand, neither the non-catechol compound soterol, which is a potent stimulant of β adrenoreceptors, nor the β -adrenoreceptor antagonist drug propranolol had any marked inhibitory effects on the binding of ^3H -noradrenaline.

It is thus clear that the structure responsible for the membrane binding of ^3H -noradrenaline is not identical with the β receptor for catecholamines. Indeed the specificity of these binding sites for catechols and their sensitivity to known inhibitors of the enzyme catechol-O-methyl transferase (pyrogallol, tropolone, quercetin) lead Cuatrecasas *et al.* to conclude that these binding sites more probably represent a membrane-bound form of this enzyme, known to exist in the microsomal fractions of the tissues studied.

The use of labelled hormones and neurotransmitters or their antagonists is likely to remain a most fruitful approach to identifying elusive membrane-bound receptor sites. But since such receptors are present in most tissues in extremely small numbers such potential labels must bind with extremely high affinity, must be available with extremely high specific activity and must obey very precise specificity rules in order for this approach to be valid. The hunting of the receptor snark can otherwise all too easily lead to boojums.

From our Neuropharmacology
Correspondent

Selective adhesion and retinotectal specificity

from a Correspondent

THE development of the brain involves the segregation of vast numbers of cells into precisely delineated subpopulations which are linked by highly selective neuronal connections. The mechanisms responsible for the formation of specific nerve connections have been analysed, most extensively in the retinotectal system of lower vertebrates: the axons of retinal ganglion cells connect selectively at local tectal sites to produce a map of the retina across the surface of the optic tectum. The most popular hypothesis that seeks to account for the discrimination in intercellular recognition that such selective connections imply is that due to Roger Sperry, who suggested, some 30 years ago, that cellular differentiation in the nervous system extends beyond the level of neuronal populations to that of the individual neurones themselves. The differentiated neurone acquires a characteristic cell surface label which participates in selective intercellular recognition and the formation of synaptic connections.

This hypothesis of neuronal specificity has withstood the test of time with remarkable success. Recent work has concentrated not so much on the hypothesis itself as on the rules by which such labelled neuronal arrays interconnect. The experimental paradigm adopted to examine the rules involves the surgical creation of relative size disparities between the innervating cell population and its target. Thus the patterns of connection that result when half a retina is caused to innervate a whole optic tectum or, conversely, when a whole retina feeds into a tectum half of which has been removed, have been described. The interpretation of such results has, however, run into a conceptual problem. One does not know whether the altered pattern of connections that is frequently found reflects altered neuronal labels, occurring as the direct result of experimental interference with a neuronal population or whether, alternatively, the neuronal labels themselves are unchanged by the operation. In this second case, the altered connection pattern might indicate the presence of a competitive mechanism in the formation of synaptic connections which are, then, determined not solely by the 'specificity labels' the neurones carry but also by the context in which the two extant neuronal populations are interconnecting (Gaze and Keating, *Nature*, **237**, 375; 1972). The inability to distinguish between these two alternatives will persist until the appearance of an experimental design in which it is possible to deduce the nature of the neuronal labels indepen-

dently of the formation of synaptic connections by the neurones.

One must await either the identification of the 'specificity molecules' or, alternatively, an assay system which reveals their presence by a criterion other than the formation of synapses. What might be the first, and very exciting, step in the direction of such an assay is provided by the very elegant experiments of Barbera, Marchase and Roth (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 2482; 1973).

Building upon earlier work which had demonstrated differences in adhesability between cells from different tissues, and from different brain regions, Barbera, Marchase and Roth decided to investigate whether there is preferential adhesion between cells which will become neuronally linked as opposed to cells that do not neuronally link. In their assay system a ^{32}P -labelled cell suspension of either dorsal or ventral retina of embryonic chicks was placed in dishes containing both ventral and dorsal tectal halves. Cells from the dorsal retina were found to adhere preferentially to ventral tectum and cells from ventral retina adhered preferentially to dorsal tectum. This preferential adhesion is similar to the innervation pattern of the retino-tectal system; dorsal retina normally connects with ventral tectum and ventral retina with dorsal tectum.

This interesting result is interpreted with appropriate caution by the authors. Indeed, some care is warranted before it can be accepted that the cell surface recognition phenomenon being demonstrated here is the one involved in specific synaptogenesis. Only a small proportion of the cells demonstrated the preferential adhesion that the authors monitored; most of the cells were easily displaced by light washing. Of those cells that did display the preferential adhesion, the difference in number between those adhering to the 'correct' tectal half exceeded those in contact with the 'wrong' half only by a factor of two, which is rather low for a system in which the differential specificity of neuronal connections is, in most models, viewed as very high.

As to the question of whether the mechanisms involved in cell adhesion are those involved in synapse formation, it must be accepted that the greatest part of the work on selective cell adhesion has emphasised the tissue-specific nature of these adhesions. Thus retinal cells selectively adhere to retinal cells, cerebellar cells to cerebellar cells (see Luis and Glaser (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 2794; 1973)). This tissue-specific aggregation probably involves quite different surface receptors from those involved in synaptogenesis, because most neuronal connections link different populations rather than cells

of the same population. In this context it is interesting to note that retinal pigment cells display the same preferential adhesion to tectal halves as do the neural retinal cells, even though retinal pigment cells are not neurones and most certainly do not form synaptic connections with the tectum.

Despite these caveats, this work of Barbera *et al.* is a most promising advance in attempts to understand the mechanisms of selective cell recognition. Such mechanisms will throw light on the formation of tissues and these experiments suggest that similar processes may underlie the elaboration of the intricate pattern of synaptic linkages that characterises the functioning nervous system.

Endemism and environmental stability

from our *Plant Ecology Correspondent*

ENDEMIC organisms and the problems underlying their origin and survival have a peculiar fascination for biogeographers. As long ago as 1882 Engler had postulated that there are basically two types of endemics, ancient ones (palaeoendemics) and those of recent origin (neoendemics). Various criteria have been used for their differentiation, the most effective being the geographic proximity of closely related species. Palaeoendemics are normally disjunct in distribution, occurring in situations well isolated from similar species. Neoendemics, on the other hand, often have many closely related species in the same or adjacent areas.

Stebbins and Major (*Ecol. Mongr.*, **35**, 1; 1965) analysed the flora of California, where roughly a third of the indigenous plants are endemic, and concluded that the palaeoendemic species are chiefly found in either markedly damp or distinctly dry environments, whereas intermediate climatic regimes are typified by neoendemics. They explain this by suggesting that palaeoendemics are found only in stable climatic situations whereas an unstable climate is conducive to rapid extinction and evolution, resulting in the production of neoendemics.

Ultimately, the demonstration that long-term climatic stability is associated with palaeoendemism is in the hands of the palaeoecologist. Meyer (*Ecology*, **54**, 982; 1973) has now accomplished this in the Cuatro Ciénegas Basin in northern Mexico, another area rich in endemics. The Cuatro Ciénegas Basin lies at an altitude of 740 m and is surrounded by mountains rising to heights in excess of 2,500 m. Many aquatic animals are endemic in the basin, including nine species of fish, three isopod genera and thirteen species of hydrobiid snails. The character of this fauna suggests that long-term isolation, per-

haps accompanied by climatic stability, has resulted in its survival and development. In other words, these organisms are palaeoendemics.

Meyer has analysed palynologically two cores from the central area of the basin in order to reconstruct the environmental history of the region. One of these cores, which was taken from a spring-head mire, reaches a depth of 13 m, and radiocarbon dating on material between 11 m and 13 m gave results of $>31,400$ BP and $>26,800$ BP. The record thus goes back to the middle of the last (Wisconsin) glacial advance, at which time the area would have been experiencing pluvial conditions.

The pollen diagrams are remarkably uniform and give no evidence of rapid climatic fluctuations. The main fluctuations are in the pollen of grasses and the *Chenopodiaceae* / *Amaranthaceae* group, which probably reflect local changes in soil salinity and hydrology rather than climatic change. Modern pollen grain studies in the region indicate that tree pollen (mainly *Pinus*, *Quercus* and *Cupressaceae*) is derived largely from the montane *Pinus ponderosa* forests and *Compositae* pollen comes mainly from the mixed xerophytic communities and creosote bush savannah of the basin floor. Since the tree pollen: *Compositae* ratio remains fairly constant throughout the profile, it is reasonable to postulate a long term stability in these major vegetation types.

In this particular locality it is therefore possible to associate a high incidence of palaeoendemism with a history of environmental stability, thus providing support for Stebbins and Major's hypothesis.

Some somatic cell variants are mutants

from a *Correspondent*

ARE somatic cell variants true mutants, that is, are they really the result of stable and hereditary changes in nucleotide sequence? This question, which surely has disturbed the sleep of many a somatic cell geneticist, receives two positive replies in the November issue of the *Proceedings of the National Academy of Sciences*.

First, Thompson, Harkins and Staners (**70**, 3094), continuing the Toronto group's work on temperature-sensitive (*ts*) variants, report that one of their *ts* Chinese hamster cells, *tsH1*, has a *ts* leucyl-transfer RNA synthetase. Mutant *tsH1* grows normally at 34° C, but after shift up to 39.5° C, cell division stops and the cells disintegrate within 24 h. The mutant shows no subclone variation, has a low reversion frequency which is increased by exposure to muta-

gens such as ethyl methanesulphonate, and is recessive in cell hybrids. The clue that *tsH1* has a primary defect in protein synthesis came when the rates of DNA, RNA and protein synthesis were examined after cells were placed at the non-permissive temperature. Both DNA and RNA synthesis decline with a half-life of between 5–8 h; notably, protein synthesis shuts off almost immediately and is down to 5% of control values by 1 h.

Following this lead, Thompson *et al.* looked for various amino-acylated tRNAs by feeding cells ^{14}C -amino acids in the presence of cycloheximide and actinomycin D. Wild-type cells and a temperature-resistant revertant showed equal amounts of leucyl-tRNA at both 34° C and 40.5° C; *tsH1* had normal amounts of all the amino acyl-tRNAs at 40.5° C, except leucyl-tRNA which was reduced more than 100 times. Direct assay *in vitro* of the leucyl-tRNA synthetase from *tsH1* showed that it is the synthetase itself which is *ts*.

The second report, by Sharp, Capecchi, and Capecchi (70, 3145), is a study of the properties of the favourite enzyme of the somatic cell geneticist—hypoxanthine phosphoribosyl transferase (HPRT)—in variants of the mouse L cell. Cells that have a normal HPRT are extremely sensitive to the purine analogues, 8-azaguanine (8AG) and 6-thioguanine; revertants can be isolated using the Littlefield technique of blocking *de novo* purine biosynthesis with aminopterin and simultaneously providing hypoxanthine and thymidine ('HAT' selection). While looking for nonsense suppressors, Sharp *et al.* examined the kinetic properties and heat stability of HPRT among such HAT-resistant revertants. Several revertants grew equally well in both medium used to isolate HPRT-negative cells (8AG) and in medium used to isolate HPRT-positive revertants (HAT). These revertants had high concentrations of HPRT.

Initially, this seems to be a surprising, if not a slightly disturbing, result, though it would be expected if the revertant enzyme had a decreased affinity to 8AG, but had the normal K_m for hypoxanthine. This is precisely what Sharp *et al.* report for one such revertant, suggesting that a structural change has occurred in the enzyme. Another class of revertants possessed a very heat-labile HPRT, again providing strong evidence of structural changes in the enzyme as the result of mutation.

These two reports, taken together with previous findings of 8AG-resistant variants that contain material that cross-reacts immunologically with HPRT but have no enzymatic activity (Beaudet *et al.*, *Proc. natn. Acad. Sci., U.S.A.*, 70, 320) and α -amanitin-resistant mutants due to an altered RNA

polymerase II (Chan *et al.*, *ibid.*, 69, 3119), should help to quiet the critics. It seems that at least some variants are mutants.

Analytical techniques for museums

from a Correspondent

THE Society for Analytical Chemistry (Midlands Region) recently cooperated with the United Kingdom Group of the International Institute for Conservation in organising a symposium at the Birmingham Museum and Art Gallery on December 4. The scope of the contributions concerned both the present types of chemical analytical methods used in museum laboratories and also reviews of techniques which, although used increasingly in chemistry research in general, may also have applications to the examination of museum objects.

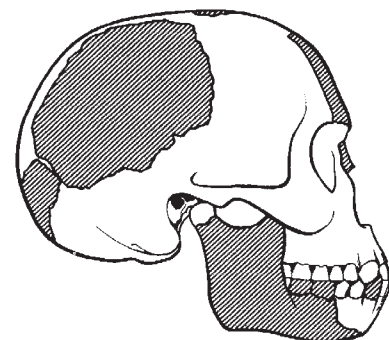
B. W. Fitzsimmons (Birkbeck College, London) described the theory and practice of Mössbauer spectroscopy and its applications to the study of ^{57}Fe and ^{119}Sn nuclei. As this technique gives information on the chemical environment of iron and tin, its potential use in museums is in the examination of clays and iron oxide pigments which may be complex mixtures of ferrous and ferric compounds. M. Thompson (University of Technology, Loughborough) dealt with photoelectron spectroscopy. Using X-ray excitation all elements except hydrogen can be detected in small samples, and it is possible to deal with many mixed oxide systems. The extreme sensitivity and high selectivity of the antigen-antibody reactions of, for example, proteins in immunological methods (J. N. Miller, University of Technology, Loughborough) which are already used in forensic science and food science suggest that they could be applied to paintings and other art work to reveal the media used by the artist. A key issue is whether antigen activity survives over long periods and this requires investigation.

After descriptions of potential new techniques, the methods used at present and results obtained in different museum laboratories were then described. J. Mills (National Gallery, London) has used infrared spectrometry and chromatography, principally GLC, to identify organic materials in art objects. Organic materials encountered in antiquities, especially paintings, are often complex natural products, but GLC of terpenoids (that is, resins) has provided new information on their identification and the chemical changes undergone over long periods. Ancient bronzes and faience in numbers running into the thousands have been analysed by H. McKerrell (National Museum of Anti-

quities of Scotland) using the version of non-dispersive X-ray fluorescence which he has developed. A rapid non-destructive method such as this has great potential for analytical surveys of large groups of museum objects. Such analyses have an important bearing on basic archaeological problems of trade contacts and sources of raw materials such as tin. In another laboratory (M. J. Hughes, Research Laboratory, British Museum) a combination of qualitative spectrographic and quantitative atomic absorption analysis has been used, because these methods are sufficiently sensitive for analyses of the small samples only which can be taken from museum objects.

Where expensive analytical equipment is not available, J. Hedley and A. Cumming (Courtauld Institute of Art, London) described how optical mineralogy, microchemical analysis and spot tests can be applied to inorganic materials used in paintings, and for organic compounds solubility tests and selective staining have been successfully applied.

Piltdown Man



THE Piltdown Man hoax still arouses curiosity and indeed was the subject of a recent British television programme some 60 years after the bits of skull and jawbone were found in a remote corner of Sussex. The British Museum (Natural History) has therefore done a service to the ordinary person who likes a scientific detective story, by producing a slim guide (*The Piltdown Man Hoax*, Palaeontology Leaflet No. 2, pp. 6; 7p) which sets out simply how the Piltdown remains came to be found, names the persons chiefly responsible for identifying the fragments, describes how the hoax was exposed at the museum and elsewhere between 1949 and 1953 and explains how, in 1973, the museum can say confidently "none of the *Eoanthropus* fragments can be considered fossil or in any way part of man's ancestry".

Weather and the Earth's magnetic field

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A comparison of meteorological pressures and the strength of the geomagnetic field suggests a possible controlling influence of the field on the longitudinal variation of the average pressure in the troposphere at high latitudes. If so, changes which occur in the pattern of 'permanent' depressions in the troposphere as the magnetic field varies (for example, as the non-dipole component of the field drifts westwards) may be accompanied by climatic changes.

I HAVE recently suggested¹ that regions of low atmospheric pressure observed near the magnetic poles occur because these regions, which are linked magnetically to the interior of the magnetotail, are strongly shielded against the incursion of solar particles. Although the mechanisms by which energetic particles may affect the troposphere are unknown, in this paper I describe the possible influence of the Earth's magnetic field on the height of the 500 mbar level in the regions surrounding the northern and southern magnetic poles. It will also be shown that certain features of the 'permanent' atmospheric pressure system seen to have moved westwards during some decades of the present century; this movement may be associated with a corresponding westward drift of the magnetic field pattern.

Figure 1 shows the average height of the 500 mbar level for January in the northern hemisphere²; the contours exhibit the typical northern hemisphere winter pattern of two lows, one near 80°W and the other near 130°E. Other examples of average pressure maps for the northern hemisphere have been published³ and they all show a highly elongated or 'dumb-bell-shaped' low at high latitudes.

Comparison with magnetic parameters

Various theories² have been advanced in order to explain the existence and location of the two lows observed in the northern hemisphere, but theoreticians working in this field do not seem to have taken into account the fact pointed out by other authors⁴⁻⁹ in the past, that the Earth's magnetic field and the behaviour of the lower atmosphere may be connected in some way.

Figure 2 contains a map¹⁰ showing contours of constant magnetic intensity, B , of the Earth's magnetic field (for middle and higher latitudes) at an altitude of 400 km in 1965; the northern hemisphere data are replotted in the polar plot shown in Fig. 3. The height for which the magnetic data are presented was chosen arbitrarily; the contours for lower alti-

tudes would not have significantly different shapes, merely different B values.

A comparison of the longitudinal variations, at 60°N, of the 500 mbar data from Fig. 1 and the magnetic B data from Fig. 2 is shown in Fig. 4. The similarity between the two curves is striking except that the magnetic- B curve is displaced about 25° towards the west. Although the meteorological data relate to an earlier epoch (1918-1958) than the magnetic data (1965), no attempt has been made to correct the magnetic data for the well-known westward drift^{11,12} of the non-dipole component of the Earth's mag-

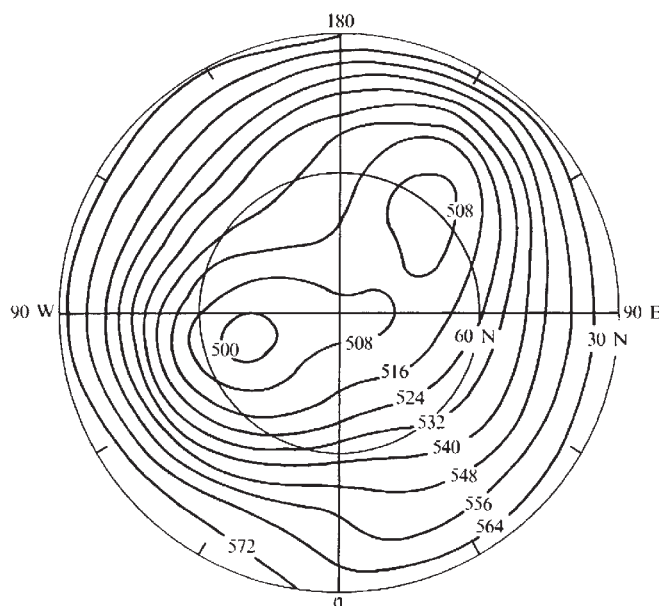


FIG. 1 Northern hemisphere polar plot showing the average height (decametres, dam) of the 500 mbar level for January. (After Palmen and Newton²).

netic field; part of the phase difference between the two curves would be removed by taking this into account. (A comparison of magnetic dip or magnetic declination maps from different epochs of the present century shows that the magnetic field patterns are rotating westward on average about 3° per decade; the drift is by no means uniform over the Earth's surface and it varies from decade to decade). The remaining phase difference may arise because of a complicated phase relationship between the atmospheric response and the modulating force imposed by the unknown magnetic-field-dependent 'driving force.'

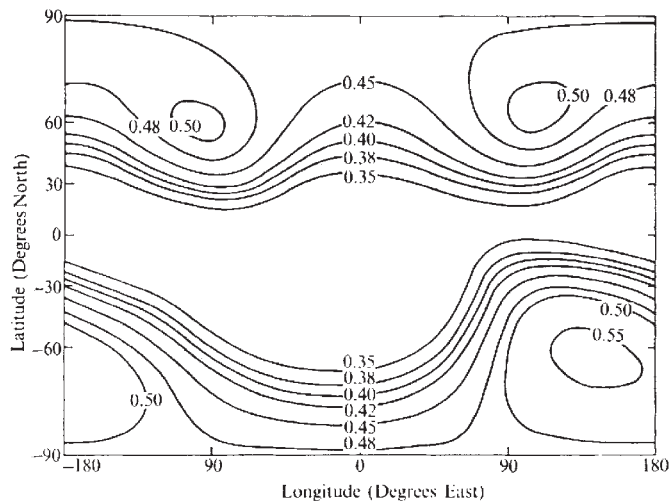


FIG. 2 Map showing contours of constant magnetic field strength B calculated for 400 km, 1965. (After Stassinopoulos¹⁰).

It should be noted from Fig. 4 that the magnitude of the 500 mbar height change associated with the magnetic field is of the order of 250 m. Comparison of the meteorological data (Fig. 1) and the magnetic data (Fig. 2) reveals that the pressure lows are centred on latitudes of 70° and 62° whereas the magnetic- B maxima occur at latitudes of 61° and 66° respectively.

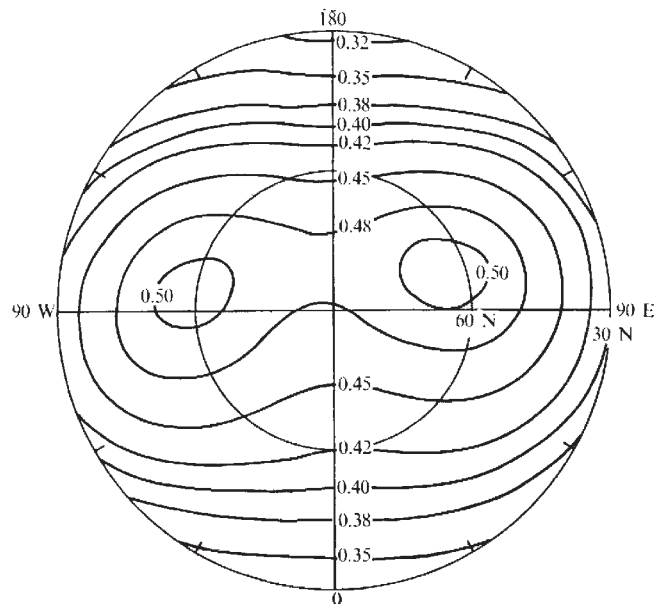


FIG. 3 Northern hemisphere polar plot showing contours, drawn from Fig. 2, of constant magnetic field strength for 400 km, 1965.

Figure 5 contains maps showing the height of the 500 mbar winter isobars over most of the northern hemisphere for epochs centred on 1933 and 1951 respectively. (The older data were obtained from ref. 13 and related to the December-February season. These data were compiled from observations reported in thirty-three documents, ten of which indicated in their titles the years for which the data applied; the average of these years was 1933. The other twenty-three documents had an average publication date of 1938 and it appears reasonable to conclude that the results are representative of years around 1933. The more recent data¹⁴ related to January during the years 1949-53).

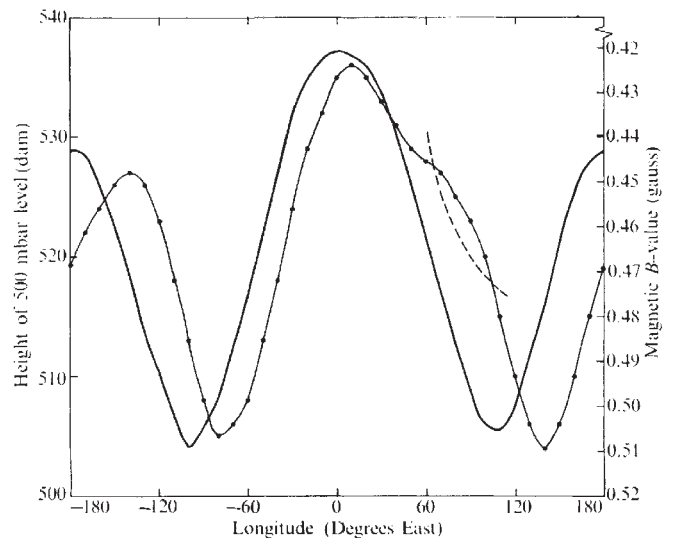


FIG. 4 Curves showing: $\bullet-\bullet$, the longitudinal variations at 60°N of the height of the 500 mbar level (from Fig. 1); $-$, the magnetic field strength (from Fig. 2). The short broken curve draws attention to some of the pressure data which may be anomalously high. The magnetic data relate to 1965 and the meteorological data to the epoch 1918-58.

Although statistically stronger evidence would be desirable, Fig. 5 does indicate that the average high-latitude pressure distribution appears to have moved westward by about 12° between 1933 and 1951; this angle is greater than that through which the Earth's magnetic field pattern rotated during the same period. Further information about the apparent westward rotation of the average pressure system is shown in Fig. 6 which relates to 60°N over the western hemisphere. Examination of the longitudes at which the maxima and minima occur shows that, averaging over summer and winter and heights between 300 and 700 mbar, the average pressure system moved about 15° westward between 1933 and 1951. It should be pointed out that the

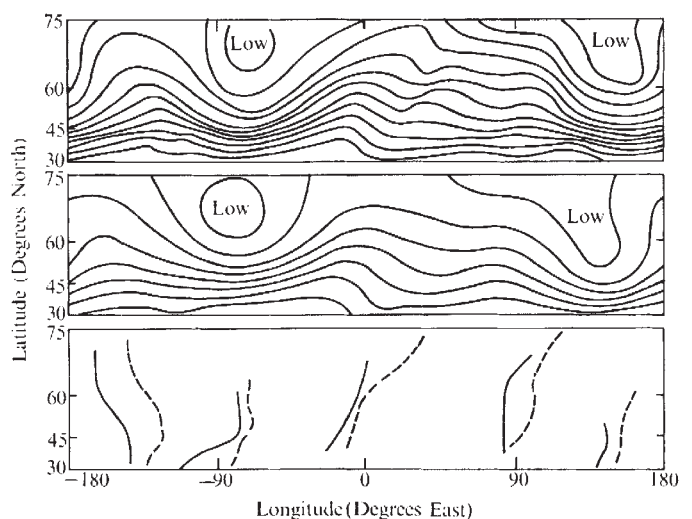


FIG. 5 Maps of the height of the 500 mbar isobars in winter. Upper: December-February, for an epoch centred on about 1933. The contours range from 500 to 567 in eleven steps of 6.1 dam. (After ref. 13). Middle: January, for the five-year period centred on 1951. The contours range from 500 to 570 in seven steps of 10 dam. (After ref. 14). Lower: Curves showing the positions of the crests and troughs appearing in the upper two sections. $- - -$, 1933; $-$, 1951.

recent data¹⁴ related only to January or July whereas the older data¹³ are for the three-month periods December–February or June–August; although significant differences exist between the average pressure distributions for different seasons, examination of all the maps available to the present author indicates that the seasonal variations cannot vitiate the conclusion that some parts of the average pressure system appear to be drifting slowly westward.

In the southern hemisphere the magnetic invariant pole is situated at a longitude of 125°E. It is well known that the geomagnetic contours surrounding the south magnetic pole are much more circular than the highly elongated ones around the northern pole and, therefore, the magnetic field should not be expected to produce meteorological effects at two longitudes in the southern hemisphere. Lamb (ref. 3, page 29) has published a polar diagram showing the mean height of the 500 mbar surface in the southern hemisphere; the low at the centre of the system is slightly displaced from the geographic pole, along the 120°E meridian. Figure 7 has

B-values in the southern hemisphere at 120°E also occur at 60°S; the height of the 500 mbar level is thus most reduced at the latitude where the magnetic field is strongest.

Implications and applications

I conclude that: (a) the Earth's magnetic field possibly influences the average tropospheric pressure system at high latitudes; and (b) the average pressure system seems to move westwards as the magnetic field rotates. The evidence relating to the second point is less convincing and there is clearly a need for further comparisons of meteorological data from widely spaced epochs for which reliable maps of magnetic field strength are also available. Even though Fig. 5 may not provide conclusive evidence about the westward motion of the pressure system, it does show that the pressure contours are very similar to the magnetic contours drawn in Fig. 2.

The large geographical extent of the permanent depressions results in considerable meridional air motion; warm air from lower latitudes at the longitude of a depression will move towards higher latitudes to the east of the depression. Clearly, if the longitude of one of the northern hemisphere depressions drifts towards the west the climate over much of the northern high-latitude region will alter. It should be noted, in this context, that the magnetic declination at London and Paris was zero in about 1660 (refs 15, 16); the magnetic field configuration then prevailing in the northern hemisphere was obviously very different from the present one and it is interesting to note, therefore, that Europe and certain other parts of the northern hemisphere experienced a "Little Ice Age"³ during the period 1550–1700 AD. It would be expected, if the average tropospheric pressure systems are indeed controlled by changes of the Earth's magnetic field, that various climatic phenomena which have occurred in the past should be associated with changes in the magnetic field. Evidence that this is so will be presented elsewhere.

The phase difference between the longitudinal pressure

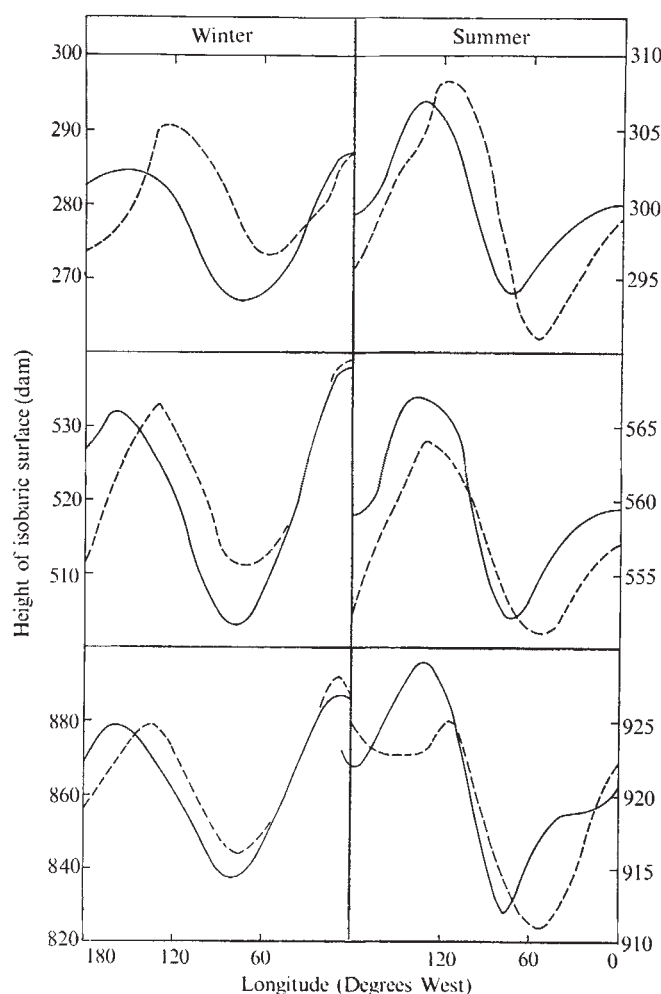


FIG. 6 Longitudinal variations of the height (dam) of the 700 (top), 500 (middle) and 300 (bottom) mbar levels in winter and summer for two epochs. — — —, 1933; —, 1951. (refs 13, 14).

been obtained from Lamb's plot and shows the latitudinal variation of the pressure down the 120°E meridian, across the geographic pole and up the 60°W meridian. The effect of the assumed magnetic-field-dependent mechanism seems to be a reduction in the height of the 500 mbar surface by a maximum of 140 m at 60°S. Figure 2 shows that the highest

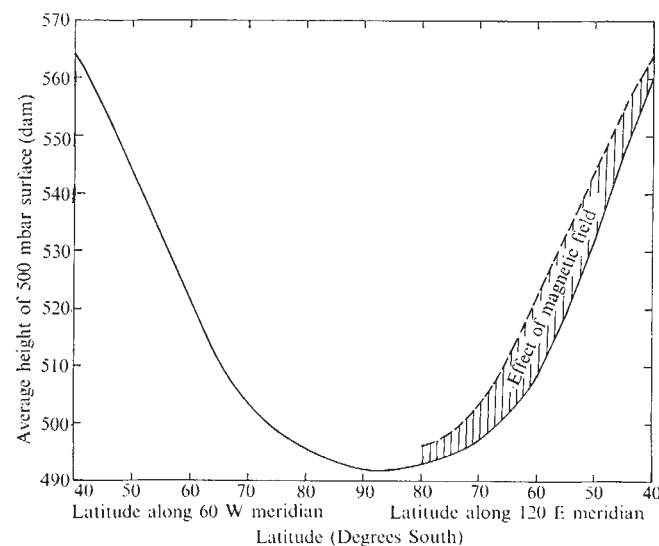


FIG. 7 Latitudinal variation of the height of the 500 mbar surface down the 120°E meridian and up to 60°W meridian. The difference between the values along the two meridians is greatest near 60°S. — Observed; — — —, mirror image of data from 60°W meridian.

and magnetic variations (Fig. 4) can only be partially explained by the westward drift of the Earth's field. I suggest that the remaining difference (between the phase of an unknown magnetic-field-dependent driving force and the response of the atmosphere) may be caused by Coriolis force

and by the inertia and viscosity of the air. If Coriolis force is important in this connection, the phase difference should be expected to vary with latitude, probably becoming greater at lower latitudes. The phase difference will also, of course, depend on the process by which energy is imparted to the atmosphere and thus the observed phase differences, when the effects of Coriolis force and so on have been subtracted, may constitute an important clue in the search for trigger mechanisms that enable changes in the solar wind or other forms of solar radiation to affect the vast mass of the lower atmosphere.

The meteorological data in Fig. 4 exhibit some irregular behaviour which is most pronounced in the longitude range 70 to 100°E, exactly the range covered by the highest mountains of Central Asia. If the longitudinal variation of the average height of the 500 mbar level at 60°N is dependent on the Earth's magnetic field, it may not be necessary to invoke orographic (related to mountains) or thermal effects associated with topographical phenomena such as land/sea boundaries to explain the major features observed. Possibly the main effect of mountains on the average height of the 500 mbar level at 60°N is the small increase, approximately 30 m, observed at the longitude of the mountains of Central Asia.

The conclusion that some unknown magnetic-field-dependent mechanism seems to modulate the tropospheric pressures by an amount which is greater in the north than in the south is particularly interesting because it is known¹⁷ that the intensity of auroral optical emissions, produced by energetic particles impinging on the atmosphere, is 30% greater in the northern than in the southern hemisphere.

It has recently been demonstrated that changes in the sector structure¹⁸ of the interplanetary magnetic field, and also changes of geomagnetic activity^{19,20}, are correlated with the depths of meteorological troughs crossing North America. Other workers²¹ have also reported that conditions in the troposphere appear to be abnormal near the times of geomagnetic disturbances. The explanation of these results may be similar to that of the marked influence which the Earth's magnetic field appears to exert on the troposphere. Some of the studies²² involving correlations between the troposphere and the sector structure have suggested why solar-weather relationships established empirically for certain periods are not observed during other epochs.

The problem of the trigger mechanism by which small amounts of energy from the solar wind can affect the atmosphere provides an interesting field of research. Roberts and Olson¹⁹ have suggested that cirrus cloud formation following ionisation produced by X-rays emitted during aurorae may be important; a difficulty about this suggestion is that auroral bremsstrahlung do not normally penetrate to tropospheric heights. Energetic solar protons and γ rays associated with heavy particles are, however, capable of reaching lower heights than the bremsstrahlung and it may be that they affect the atmospheric pressure in some way. It is known, alternatively, that changes in the solar radiation affect the stratospheric ozone concentration^{23,24} and perhaps, therefore, the thermal balance of the lower atmosphere.

It seems possible that the solar wind may influence the behaviour of the Earth's atmosphere if the accretion of solar wind protons results in the production of water vapour in the stratosphere^{25,26}. De Turville²⁵ suggested that all the water in the oceans might have been formed in this way, but concluded later²⁷ that this was not the case; the considerations which led to the withdrawal of the suggestion involved the rate at which water vapour would be produced rather than whether the process occurred at all. Wasson and Junge²⁸ concluded: "The importance which is attached to an extra-terrestrial origin for even part of the stratospheric water-vapour means that water-vapour measurements, especially in the polar stratosphere, could be of great

scientific value". It is well known that the penetration of the solar wind to the near-environment of the Earth depends on the terrestrial magnetic field and it would seem worth examining, therefore, whether the spatial distribution of water vapour in the stratosphere, and hence the thermal balance of the lower atmosphere, is also dependent on the field. If the relatively low atmospheric pressures that occur in regions of high magnetic intensity are a consequence of shielding by the magnetic field against direct injection of certain solar wind particles it should be expected that, since the angle between the magnetic dipole and the solar wind is very different in summer from that in winter, solar-weather relationships will show marked seasonal changes. Roberts and Olson²⁰ have reviewed evidence which clearly shows that this is the case.

Although the evidence presented in this paper cannot be construed as proof that the Earth's magnetic field and the average tropospheric pressure patterns are related, it does suggest that more work on this problem should be undertaken.

I propose that the field of research involving inter-relationships between meteorological phenomena and processes depending on the Earth's magnetic field should be known as 'magnetometeorology'.

I thank Drs W. C. Bain, H. H. Lamb, J. A. Saxton and D. M. Willis for comments and Dr D. L. Croom for drawing my attention to the stratospheric water vapour problem²⁵⁻²⁸.

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Myosin from Cross-reinnervated Cat Muscles

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Correlated physiological changes, and structural and functional changes in myosin, following cross-reinnervation of cat fast-twitch and slow-twitch muscles, indicate that the myosin phenotype is controlled through the nervous system.

THE changes which occur in the isometrically recorded contractions of cross-reinnervated mammalian fast-twitch and slow-twitch muscles are now well documented¹⁻³. In many respects, the study of isotonic shortenings can, however, be more directly informative about changes in the contractile machinery. Close, using the rat^{2,4}, first clearly demonstrated that cross-reinnervation of the soleus (SOL, slow-twitch) and extensor digitorum longus (EDL, fast-twitch) muscles produced reciprocal changes in both the maximum speed of shortening (V_0) and the curvature of the force-velocity relationship. More recently Bárány and Close⁵, using the rat, have shown that the activity of the Mg^{2+} -dependent actomyosin ATPase also changes following cross-reinnervation, in a manner which parallels the alteration in V_0 . These findings supported Bárány's earlier hypothesis⁶ that the actomyosin ATPase activity is the rate limiting step which determines a muscle's maximum shortening velocity.

Isotonic studies of cross-reinnervated cat muscles have given less clear cut results than in the rat. While cross-reinnervation of fast-twitch muscle by a nerve formerly innervating soleus (a slow-twitch) muscle produced a marked reduction in V_0 , as in the rat, cross-reinnervation of the soleus muscle produced a variable but usually small increase in V_0 (ref. 7). Studies of the muscle myosin Ca^{2+} -dependent ATPase activity showed this to parallel the changes in shortening velocity⁸.

A further study⁹ of the isotonic shortenings of cross-reinnervated cat muscles using soleus as the slow-twitch muscle but the larger head of the deep digital flexors, correctly called flexor hallucis longus (FHL) rather than the formerly used smaller head (flexor digitorum longus, FDL)⁷ as the fast-twitch muscle, has led to results which are in general agreement with those obtained in the rat. In particular, following cross-reinnervation, the soleus muscle showed a marked increase in V_0 , a change which was accompanied by a decreased curvature of the force-velocity relationship. It was then of interest to know whether, as in former cases, the ATPase activities of the myosins from these muscles mirrored their shortening velocities, and further whether the myosin light chains were altered following cross-reinnervation. These low molecular weight proteins, which are part of the enzymatically active "head" of the myosin molecule, are chemically different in normal fast-twitch and slow-twitch

muscles and their electrophoretic mobilities provide a characterisation of the myosin phenotype¹⁰⁻¹².

The results described in this paper were obtained from the muscles of four cats which had been cross-reinnervated for approximately 12 months and which formed part of a larger physiological study⁹. The four animals were selected because they showed a purity of reinnervation by the crossed nerve in excess of 90%. As the muscles were required for biochemical studies, histological examination was not undertaken, the success of the cross-innervation being gauged by the purity of the reinnervation and the fact that on indirect stimulation the cross-reinnervated muscles developed a mean of 91% of the maximum isometric tension of the control muscles. It was noted, however, that the cross-reinnervated soleus muscles tended to develop somewhat more tension than their controls and the cross-reinnervated FHL muscles less.

The apparatus and methods used have been described fully elsewhere¹³. Fibre lengths were measured by the method of Al-Amood and Pope¹⁴. At the end of the physiological experiments the muscles were excised, blotted and weighed, then frozen in liquid nitrogen and stored at -20°C .

Myosin was prepared essentially as described by Bárány and Close⁵. The muscles were chopped finely with a razor blade and connective tissue removed, then homogenised in 30 mM KCl, 5 mM potassium phosphate at pH 6.7. The myofibrils were pelleted by centrifugation at 10,000g and the supernatant used to measure the absorbance of the Soret band at 417 nm (for quantitation of haem proteins). The myofibrils were washed twice more with 20 volumes of the same buffer, then extracted in a medium containing 0.3 M KCl, 0.15 M potassium phosphate, 5 mM MgATP, 0.1 mM dithiothreitol at pH 6.6 for 20 min at 4°C with gentle stirring. The extract was centrifuged at 27,000g for 10 min and the supernatant dialysed overnight against 30 mM KCl to precipitate the myosin, which was collected by centrifugation at 10,000g for 15 min. The myosin precipitate was washed with 30 mM KCl to aid removal of excess nucleotides, dissolved at 0.6 M KCl, 20 mM potassium phosphate at pH 6.7 and clarified by centrifugation at 40,000g for 30 min. Myosin was reprecipitated by dialysis against 30 mM KCl, 2 mM potassium phosphate and finally dissolved in 0.6 M KCl, 20 mM potassium phosphate at pH 6.7 and clarified once again.

The myosin concentration was estimated by absorbance at 280 nm assuming an extinction coefficient that obtained for rabbit fast muscle myosin ($E_{280}^{1\%} = 5.60$ (ref. 15)). The extinction coefficients measured for myosins prepared as described from frozen cat muscles were up to 25% higher than this value and SDS gels showed the presence of contaminating actin, which could not be completely removed without unacceptable loss of myosin. For this reason, in some preparations the extracted myofibrils were centrifuged at 165,000g for 4 h to pellet contaminating actin. The myosin was precipitated by dialysis against low salt, redissolved in 0.6 M KCl, 20 mM potassium phosphate at pH 6.7, and subjected to a second ATP dissociation followed by centrifugation at

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165,000g for a further 4 h to remove residual actin. Finally, the myosin was reprecipitated by dialysis, the precipitate washed to remove nucleotide and redissolved as above. These preparations, which were largely free of actin, gave extinction coefficients which were the same for both the SOL and FHL myosins ($E_{280}^{1\%} = 6.2$, based on the Folin reaction using bovine serum albumin as standard). The need for accuracy in protein determination could be circumvented by the use of stopped-flow assays, which are independent of protein concentration. The molecular weight of myosin was taken as 470,000¹⁶, and the molarity based on two equivalent structure units of 235,000 daltons. F-actin was prepared according to Kendrick-Jones *et al.*¹⁷.

Solvent conditions for the different kinetic assays of the ATPase are described in Table 1 and Fig. 1. The steady state actin-activated Mg^{2+} -dependent ATPase was used most extensively. The Mg^{2+} -dependent ATPase was measured occasionally but required relatively large amounts of myosin. A further assay measured the rate of protein fluorescence increase when myosin was mixed with a large molar excess of ATP, using the stopped-flow fluorimeter technique of Bagshaw *et al.*¹⁸. With the experimental conditions used, the observed rate constant of this pseudo-first order reaction is directly proportional to the ATP concentration and independent of myosin concentration. This method has a second advantage that the observed rate is independent of small amounts of contaminating actin and thus two potential sources of error in comparing different myosins are eliminated. The percentage increase in fluorescence when ATP was mixed with fast-twitch and slow-twitch muscle myosins was taken to be the same, that is, $8 \pm 2\%$ (Fig. 1).

Polyacrylamide gel electrophoresis in the presence of SDS was carried out according to Weber and Osborn¹⁹. The samples were mixed with a five-fold excess of SDS by weight and 1% β -mercaptoethanol and placed in a boiling water bath for 3 min. Aliquots of 50–150 μ g were applied to 7 cm gels. Staining was in 0.2% Coomassie Brilliant Blue in 50% methanol, 10% acetic acid for 2 h at 45° C and the gels were destained initially in the same solvent and then in 5% methanol, 10% acetic acid also at 45° C.

Twitch time

The isometric contraction data were similar to those reported earlier^{1,3,9} and showed that the twitch myogram of a cross-reinnervated FHL muscle slowed so that it came to resemble that of a normal or self-reinnervated soleus muscle and *vice versa*.

As has been reported previously³, the increase in the twitch time of cross-reinnervated fast-twitch muscles is more marked than the decrease in the twitch time of cross-reinnervated soleus muscles, and this observation was confirmed in the present series with one exception. The cross-reinnervated soleus muscle of the experiment of May 18 showed a more marked speeding of its isometric twitch time than we have seen in any animal before or since. The normal (right) soleus of this cat had a contraction time of 87 ms and the cross-reinnervated (left) a contraction time of 25 ms. Because of the unexpectedness of this observation physiological studies were concentrated on the soleus muscles of this cat, and isotonic results were not obtained on either the normal or the cross-reinnervated FHL muscle.

The isotonic contraction results showed a reduction in the maximum shortening velocity of cross-reinnervated fast-twitch muscles as has been reported earlier⁷. But, contrary to this same report⁷, we observed in the present experiments (and again particularly in the cat of May 18) a marked increase in the maximum shortening velocity of the cross-reinnervated soleus muscles (see Tables 1 and 2).

Table 1 Characteristics of Normal and Cross-reinnervated FHL and SOL Muscles from May 18 Cat

	N-FHL	X-FHL	N-SOL	X-SOL
* TP (ms)	29	54	87	25
† V_0	—	—	4.82	11.6
‡ Soret band	0.50	0.99	1.0	0.56
§ Rate of fluorescence change (Fig. 1) (s^{-1})	5.6 (3.3)	2.0 (1.2)	1.7 (1.0)	5.0 (2.9)
Mg^{2+} -ATPase (s^{-1})	0.053 (2.9)	0.019 (1.05)	0.018 (1.0)	0.035 (1.95)
¶ Actin-activated Mg^{2+} -ATPase (s^{-1})	2.7 (10.0)	—	0.27 (1.0)	2.5 (9.3)

* TP, time to peak of isometric twitch (the time from the start of contraction until peak tension development).

† V_0 , maximum unloaded shortening (muscle fibre lengths s^{-1}).

‡ Soret band, relative absorption (λ_{max} 417 nm) of haem proteins in the first extract of the blended muscle (see preparative procedures)^{28,29}.

§ Rate of fluorescence change was the observed rate when 12.5 μ M ATP was mixed with the myosins as illustrated in Fig. 1.

|| Mg^{2+} -dependent myosin ATPase activity using 2.5 mg ml^{-1} myosin measured in 0.6 M KCl, 5 mM $MgCl_2$, 50 μ M dithiothreitol, 0.5 mM EGTA at pH 8.0 and 25° C initiated by addition of 0.4 mM ATP, using a radiometer pH-stat.

¶ Actin-activated Mg^{2+} -dependent myosin ATPase activity using 0.02–0.2 mg ml^{-1} myosin measured in 0.67 mM ATP, 23 mM KCl, 1 mM $MgCl_2$, 6.7 μ M $CaCl_2$ at pH 8.0 and 22° C in a radiometer pH-stat, initiated by addition of 0.85 mg ml^{-1} actin. Insufficient X-FHL myosin was available to obtain a reliable actin-activated Mg^{2+} -ATPase activity, but such results as were obtained indicated the activity was comparable to that of the normal soleus myosin. The figures in brackets are values relative to the normal soleus.

Myosin activity

Before studying myosins from the cross-reinnervated muscles several control experiments were carried out on preparations of fast-twitch and slow-twitch myosins from normal cat muscles. ATPase activities of myosins isolated from fresh muscles were identical to those of myosins isolated from corresponding muscles stored at -20° C.

A further control experiment concerned the extractability of myosins from different muscles. Normal FHL and soleus muscles well cleaned of connective tissue were mixed in known proportions by weight (3:1, 2:2, 1:3) and the resultant myosins isolated. Kinetic analysis of the pure myosins and the mixtures gave activities which showed that each myosin was extracted essentially in proportion to its original content in the muscle (that is, the myosin from the 2:2 muscle mixture behaved as an equal weight mixture of pure FHL and soleus muscle myosins). This is an important result, since the yields of myosin from the muscles were variable and often rather low, particularly the cross-reinnervated FHL muscles. These low yields occurred with smaller muscles which seemed to contain a proportionally higher content of connective tissue, and whose myosins precipitated less quantitatively in low salt because of their relatively low concentrations.

The kinetic results from the myosins of the May 18 cat are summarised in Fig. 1 and Table 1. (N-FHL, S-FHL, and X-FHL are respectively normal, self-reinnervated and cross-reinnervated flexor hallucis longus muscles; N-SOL, S-SOL and X-SOL, normal, self-reinnervated and cross-reinnervated soleus muscles.) They show that cross-reinnervation has resulted in almost complete cross over from the kinetic viewpoint. This can clearly be seen in Fig. 1 when the observed rates are dependent solely on the type of myosin and not on its total concentration.

The physiological measurements indicated that in other experiments transformation was less complete and this was reflected in the actin activated Mg^{2+} -dependent ATPase

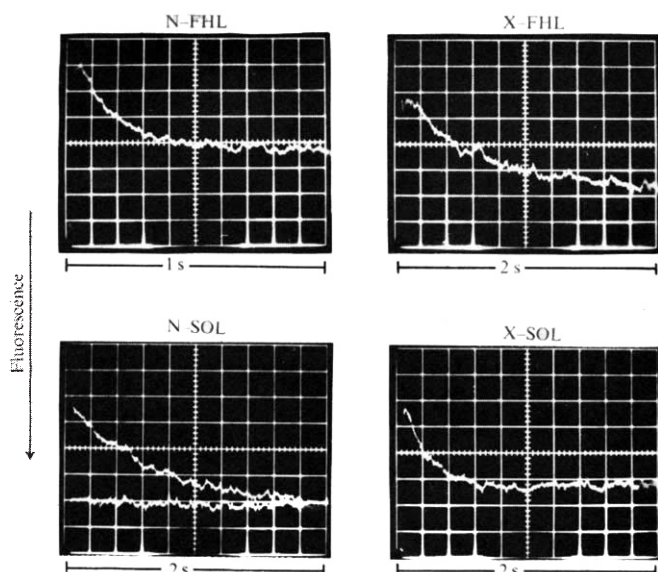


Fig. 1 Stopped-flow spectrophotometric record of the protein fluorescence increase when ATP is mixed with myosin isolated from normal and cross-reinnervated FHL and soleus May 18 cat muscles at 22° C. One syringe of the stopped-flow apparatus contained myosin (0.50 mg ml⁻¹), 2.1 μM in myosin 'heads' and the other 12.5 μM ATP. Both syringes contained 0.5 M KCl, 5 mM MgCl₂, 0.1 mM dithiothreitol, 0.2 mM EGTA and 50 mM Tris adjusted to pH 8.0 with HCl. Reaction chamber concentrations are recorded. The horizontal trace on the N-SOL myosin photograph was recorded a few seconds after the reaction was initiated and shows the protein fluorescence during steady state ATPase activity. Observed first order rate constants were obtained from the oscilloscope traces, and the mean values of a number of records for each myosin are listed in Table 1. The fluorescence stopped-flow apparatus, built by Professor H. Gutfreund and Dr D. W. Yates, has been described previously¹⁸. The protein was excited by light at 300 nm and the emitted light between 335 and 375 nm analysed. Measured in this way the percentage increase of protein fluorescence when ATP bound to myosin was 8 ± 2%.

activities (Table 2), where the myosin from cross-reinnervated soleus muscle had ≤50% of the control (self-reinnervated) FHL activity. A striking feature of our results is the high activity ratio between the control FHL and soleus myosins from the May 18, August 30 and September 18 cats. (Compare, for example, ratios of approximately 2:1 obtained by Bárány and Close⁵.) A lower ratio was obtained from the August 23 cat, where assays were carried out at higher ionic strength and lower actin concentrations. It was important to check whether the assay conditions were close to $V_{\max}$ for both proteins with respect to actin. This was shown to be the case by measuring the ATPase activity at several actin concentrations between 0.07 and 1.3 mg ml⁻¹, using the control myosins from the August 30 cat. The S-FHL activity showed a plateau above 0.3 mg/ml actin, while the S-SOL activity was >90% of $V_{\max}$ at 1.3 mg ml⁻¹ actin. Thus the use of low ionic strength conditions and saturating actin concentration at 25° C produce activity ratios in excess of 10:1 for normal fast-twitch and slow-twitch muscle myosins.

Myosin light chains

Both the physiological and kinetic experiments show that cross-reinnervation can produce a reciprocal transformation of cat fast-twitch and slow-twitch muscles (Table 2), and the myosins were further examined by SDS gel electrophoresis to show the light chain distribution. Figure 2 shows the results for the May 18 cat. The positions of the three light chains characteristic of fast-twitch muscle myosin are shown by the rabbit light chain marker. The myosins from the N-FHL and

X-SOL seem identical, so too do the myosins from the S-SOL and X-FHL, though here the number of bands observed in the light chain region is higher than expected. (Compare results with refs 11 and 12 for slow-twitch muscle myosins of the rabbit.) Attempts to eliminate some of these components by further purification of the myosins either by ammonium sulphate precipitation or on DEAE-cellulose columns in pyrophosphate buffers²⁰ did little to remove the additional

Table 2 Characteristics of Muscles and Myosins from Cross-reinnervated Cats

	S-FHL	X-FHL	S-SOL	X-SOL
August 23 cat				
TP	30	51	75	37
V_0	16.3	6.51	6.27	11.1
Actin-activated Mg ²⁺ -ATPase (s ⁻¹)	0.72	0.31	0.19	0.37
Physiological conversion		0.96		0.48
Biochemical conversion		0.77		0.34
August 30 cat				
TP	24.5	52	77	39
V_0	10.8	7.28	6.45	8.43
Actin-activated Mg ²⁺ -ATPase (s ⁻¹)	3.86	0.83	0.20	0.88
Physiological conversion		0.81		0.46
Biochemical conversion		0.83		0.35
September 18 cat				
TP (ms)	31	58	95	45
V_0	13.6	7.58	5.07	8.34
Actin-activated Mg ²⁺ -ATPase (s ⁻¹)	2.12	0.71	0.20	0.50
Physiological conversion		0.71		0.38
Biochemical conversion		0.74		0.16

Definitions of TP and V_0 are given in the legend to Table 1. The physiological and biochemical conversions are estimates of the degree of conversion⁸ calculated from the V_0 and actin-activated ATPase activities respectively. The physiological conversion for

$$\text{soleus} = \frac{[V_0(\text{X-SOL}) - V_0(\text{S-SOL})]}{[V_0(\text{S-FHL}) - V_0(\text{S-SOL})]}$$

$$\text{and for FHL} = \frac{[V_0(\text{S-FHL}) - V_0(\text{X-FHL})]}{[V_0(\text{S-FHL}) - V_0(\text{S-SOL})]}$$

For the September 18 and August 30 cats the actin-activated Mg²⁺-dependent ATPase activities were measured using 0.2–1.6 mg ml⁻¹ myosin under conditions described in Table 1. The actin concentration was 1.3 mg ml⁻¹ throughout. The KCl concentration in the experiments with the August 30 cat was 19 mM instead of 23 mM. For the August 23 cat the assays were carried out using a 2:1 (w/w) ratio of actin to myosin and the assay conditions were 30 mM KCl, 2 mM MgCl₂, 0.5 mM ATP at pH 7.6.

bands, though the two fastest bands (Fig. 2e, f) were somewhat decreased in intensity. Purification by ion exchange chromatography was not used routinely because of the considerable loss of protein during the fractionation and subsequent concentration procedures. In spite of this marked heterogeneity in the light chain band pattern, the myosins from N-SOL and X-FHL were indistinguishable from each other in the light chain region and showed significant differences when compared with the myosins from S-FHL and X-SOL muscles. Similar observations were made for the other cats, showing the cross-reinnervation results in the production of myosins whose light chain mobilities on SDS gels are consistent with a cross over of myosin isoenzyme production.

The results described above show a correlation between the physiological changes that accompany cross-reinnervation of cat fast-twitch and slow-twitch muscles and both the kinetic and structural properties of the myosins obtained from these muscles. They confirm and amplify the results of Bárány and Close⁵ with the rat and Samaha *et al.*²¹ and Buller *et al.*⁸ with the cat. In particular the experiment of May 18 demonstrates that in the cat an effectively complete cross over can occur not only in the actin activated Mg²⁺-dependent ATPase activi-

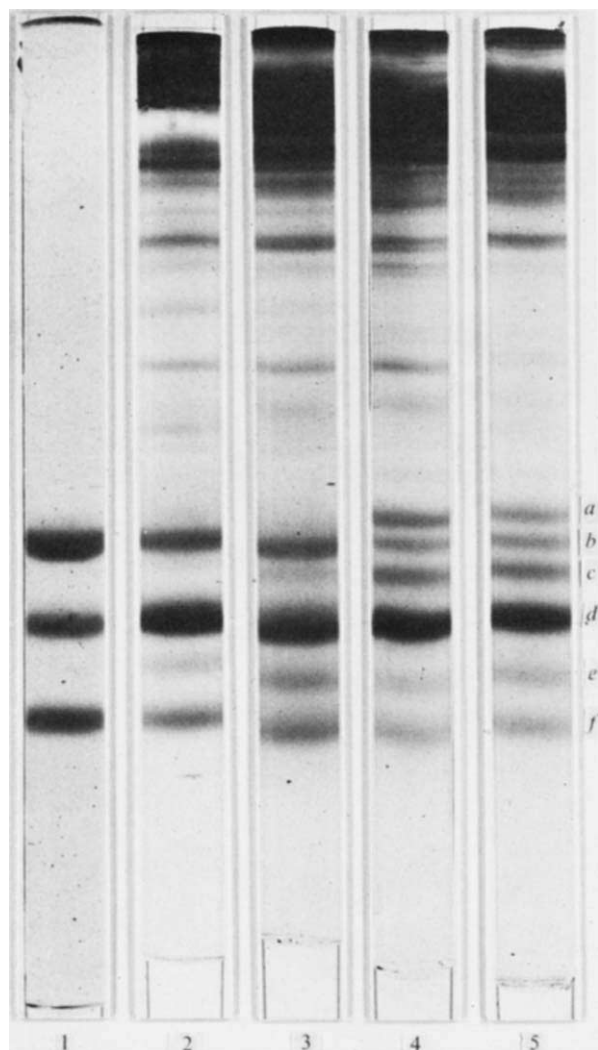


Fig. 2 Polyacrylamide gel electrophoresis of myosins from the May 18 cat run in the presence of SDS on 10% gels. 1, Rabbit myosin light chains; 2, N-FHL; 3, X-SOL; 4, N-SOL; 5, X-FHL. The letters *a-e* denote the light chain bands in the slow-twitch muscle myosin.

ties of the myosins (see ref. 8), but also in the light chain pattern observed by SDS gel electrophoresis of the myosins.

The biochemical methods used in this work for estimating actin-activated ATPase activities have differed somewhat from those described previously^{5,8}. Other authors have attempted to retain conditions approximating those found *in vivo* and the actomyosin composition has reflected that observed in myofibrils, whereas we have used techniques to exhibit the maximum differences between the myosins in a variety of kinetic assays which are nevertheless related to important activities *in vivo*. Although attempts to mimic the *in vivo* situation inevitably fall far short of exactness, we see no reason to prefer one set of data to the other. On the other hand we would certainly not claim that our kinetic techniques provide a more precise indication of the *in vivo* situation. In particular we see nothing in the present results to invalidate the Bárány hypothesis⁶, though the failure to obtain quantitatively similar ratios for the physiological and biochemical conversions (see Table 2) suggests that factors other than myosin ATPase activity may also play a part in determining a muscle's maximum shortening velocity. Previous reports^{5,8}

have also contained data on the Ca^{2+} -dependent ATPase activities of the myosins from cross-reinnervated muscles and similar assays were carried out on our myosin preparations, but the results did not show such dramatic differences between the fast-twitch and slow-twitch myosins as are recorded in Tables 1 and 2, and the cross-reinnervated muscle myosins were always intermediate in value between the control activities. This kinetic assay is particularly sensitive to modification of the myosin, for example, through thiol oxidation, and we are inclined to agree with Bárány and Close⁵ that it is a less reliable method for distinguishing myosins from cross-reinnervated muscles. Furthermore, this assay has no physiological significance since the role of calcium *in vivo* is regulatory²².

The complexity of the light chain gel pattern observed in Fig. 2 for cat myosins is greater than that reported for rabbit myosins. Both N-FHL and X-SOL myosins show additional minor bands when compared with rabbit light chains. Control preparations of rabbit soleus myosin show two bands whose mobilities correspond to molecular weights of 27,000 and 18,000^{11,12,23} and our observations agree with these. Large scale preparations of myosin from normal cat soleus muscles have, however, always given a pair of bands in the 27,000 dalton region corresponding to *a* and *b* in Fig. 2, together with a strong third band equivalent to *d*. Increased heterogeneity in the light chain region invariably occurred when the light chains were isolated from cat soleus myosin, yielding the additional bands (*c*, *e* and *f*) seen in Fig. 2 for the N-SOL and X-FHL myosins. These additional bands probably arise as a result of proteolytic degradation of the cat myosin.

The significance of the gels shown in Fig. 2 is to demonstrate the overall similarity between myosins from N-SOL and X-FHL muscles and to distinguish these from the N-FHL and X-SOL myosins. This can be most easily appreciated by the presence or absence of band *a*, which is characteristic of slow-twitch muscle myosin. Bands *b*, *d*, *e* and *f* cannot be distinguished by their mobilities alone from corresponding bands in the fast-twitch muscle myosins and, although the presence of bands *e* and *f* in N-SOL and X-FHL myosins may be attributed to proteolytic degradation, the presence of both myosin isoenzymes in the X-FHL cannot be ruled out. Unequivocal assignment of the proteins in these bands can only be made after further analysis of the light chains involved, and current studies on the thiol peptides present in the different light chains should provide unambiguous evidence for the identity of each component.

Other observations indicate that it is not only the light chain components that change following cross-reinnervation. Evidence has been adduced from the content of methylated histidine and lysine that the heavy chains of myosin also change in response to neural influence, and these preliminary studies²⁴ are supported by gel electrophoretic studies of heavy meromyosins produced from cross-reinnervated rat muscles²⁵.

All these results indicate that the myosin phenotype is controlled by the nervous system. At the present time it is not clear whether this control is exercised exclusively by the pattern of nerve impulses reaching the muscle, or whether other factors such as a trophic (non-impulse) influence also play a part. That the former is of considerable importance has recently been demonstrated by experiments in which myosin light chains characteristic of slow muscle have been synthesised in response to long-term stimulation of rabbit fast-twitch muscle²⁶. It has also been shown that long-term stimulation of cat fast-twitch muscle results in a marked decrease of the muscle's maximum shortening velocity²⁷. These observations, together with the present results, certainly suggest that if an altered activation of muscle occurs following cross-reinnervation it might play a major role in the mechanical and biochemical changes which occur. It would be interesting to know if the electromyogram activity of muscles does change following cross-reinnervation.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Spectroscopic Variations in Cyg X-2

THE X-ray source Cyg X-2 has been optically identified with a fifteenth magnitude blue object¹. Initial spectroscopic observations²⁻⁴ revealed the presence of broad Balmer absorption lines, a narrow, strong Ca II K line and a narrow emission line of He II $\lambda 4,686$. Radial velocity variations were evident, with the indication that the emission and absorption line velocities varied 180° out of phase. Photometric monitoring⁴ showed the object to vary erratically on a time scale of minutes. Later spectroscopic observations^{5,6} confirmed the changes in radial velocity, but no certain evidence of binary motion could be demonstrated. A more recent spectroscopic observation⁷ showed the He II emission to have developed a P-Cygni profile, and emission at $\lambda\lambda 4,640-50$ due to C III-N III was present. I report here the results of additional spectroscopic observations of this object.

Spectrograms were obtained on 1972 July 13.340, 1972 July 14.417 and 1973 July 9.367 UT with the 82-inch (207-cm) and 107-inch (272-cm) reflectors at McDonald Observatory. The first two spectra cover the wavelength region from $\lambda\lambda 4,400$ to 6,700 at a dispersion of $210 \text{ \AA mm}^{-1}$. The third spectrogram covers the wavelength region from $\lambda\lambda 3,800$ to 5,700 at a dispersion of $115 \text{ \AA mm}^{-1}$. Both 1972 spectra show the He II and C III-N III emission features and P-Cygni structure is evident in the helium line. The radial velocity of the He II emission is -400 km s^{-1} for both spectra. This same velocity was observed on a spectrogram obtained the previous year⁷.

The spectrogram obtained in July 1973 shows a strong Ca II K line, indicating a spectral type of F5 or later. The strength of the Balmer absorption lines, however, indicates a spectral type of A5 or earlier. The C III-N III emission is no longer present. The He II emission line is present, but with no evidence of P-Cygni structure. The measured radial velocity of the He II feature is -390 km s^{-1} . It seems that Cyg X-2 no longer exhibits large radial velocity variations. It should be

noted that Cyg X-2 was monitored photometrically in 1971 and 1972 and no rapid variability was seen (P. Vanden Bout, private communication). The above data and the report⁸ of radio variability in Cyg X-2 suggest that further optical studies are sorely needed.

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Tungus Event Revisited

THE suggestion of Jackson and Ryan¹ that a "mini" black hole was responsible for the Tungus Event of 1908 is both imaginative and intriguing. Unfortunately, this miniature, hypothetical object cannot account for all the important phenomena known to accompany the event. On the same night of the Tunguska Fall, and for several subsequent nights, the Tungus and adjacent regions experienced abnormally bright skies². Inhabitants of the entire Northern Hemi-

sphere recorded anomalous extinction of atmospheric light subsequent to the fall².

Investigation of the soil samples in the Tungus area revealed numerous small magnetic globules with high nickel content. Although the high nickel content affirms that the globules are of extraterrestrial origin, this information alone does not insure that they originated with the Tungus Event. Most soil samples collected at random over the globe will contain similar cosmic dust. The spatial pattern of the globules collected in the Tungus, however, shows that the cosmic dust most likely originated from a massive meteoritic body of dimensions vastly greater than a few Angstroms. Furthermore, this body had a trajectory similar to that of the Tunguska meteorite³. In addition to the magnetic globules, field workers in the area found fused silicate-magnetite spherules near the Tunguska epicentre. This type of cosmic remnant has been found nowhere else in the world⁴.

All these data support the hypotheses that the Tunguska Event was caused by a comet with a head composed of iron and silicate fragments cemented together by frozen volatiles. On contact with the Earth's atmosphere, the comet exploded, scattering dust and fragments over the landscape and into the atmosphere, leaving no significant crater.

Although other phenomena may possibly be invoked to account for the Tunguska meteorite, it seems that the black hole is eclipsed by a comet. Of course this discussion does not preclude the possibility that a black hole comprised the nucleus of the comet or that black holes frequently may be the agent's condensing the materials in such bodies.

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Generalisation of Hubble's Law

RECENTLY, several new efforts to improve the upper bound on the possible rest mass (m_0) of the photon have been made¹⁻³. The theoretical basis is provided by Proca's equations¹

$$(\square + \mu^2)A_K = (4\pi/c)J_K \quad (1)$$

where charge is assumed conserved and $\mu = m_0c/h$. The bounds vary from 2×10^{-43} g to 3×10^{-56} g. As Yourgrau and Woodward⁴ point out, however, a finite mass is implicit in a 'tired light' interpretation of the cosmological redshift. When equation (1) is combined with Hubble's law⁵

$$dv/dl = -Hv/c \quad (2)$$

it yields $\mu = H/2c$ and hence $m_0 < 10^{-63}$ g. Here v is the photon frequency, l the distance traversed and H is Hubble's constant. In view of this limit a direct demonstration of the existence of a finite photon rest mass does not seem practical at present.

Photon-photon interaction has been suggested⁶⁻⁸ as the 'tired light' mechanism involved. Although this does imply a finite photon rest mass serious objections have been raised on both experimental⁹ and theoretical^{10,11} grounds.

A direct local laboratory measurement of a 'tired light' effect requires a precision of about 1 part in 10^{18} per light s. That is well beyond present capabilities. As we shall indicate, however, equation (2), when generalised, implies a corollary 'tired body' effect which may be observable in a local system.

To see this, one has only to multiply equation (2) by Planck's constant (h).

$$d(hv)/dl = d(hv/c)/(dl/c) = -H(hv/c) \quad (3)$$

This immediately expresses the change of both the momentum and the energy of the photon as it moves along its path. Both of these relations can be combined into the four-vector form which constitutes the generalised Hubble's law (see ref. 12; a restricted form of equation (4) is given here).

$$dP_K/d\tau = -HP_K \quad (4)$$

The space terms yield the loss of momentum per unit time and the fourth component the loss of energy per unit length. Thus it seems from equation (4) that a non-relativistic body moving with momentum $\vec{P}$ will experience a 'tired body' force.

$$\vec{F} = -H\vec{P} \quad (5)$$

Since bodies in linear motion present the same observational difficulties as does light we turn to rotational motion. From equation (5) we see that the rate of loss of angular momentum $\vec{L}$ in a rotating system is given by

$$\vec{L} = -H\vec{L} \quad (6)$$

As an application, we can take the Earth-Moon system. On the basis of fossil data we have shown that its angular momentum has apparently been continually lost over the past 1.75×10^9 yr¹³. The total loss over this period is about 5% of the present value. Calculation of H from equation (6) gives a value of about 10^{-18} s⁻¹ as compared to the latest reported value¹⁴ of $1.3^{+0.4}_{-0.4} \times 10^{-18}$ s⁻¹. Details of this calculation are given elsewhere¹².

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BIOLOGICAL SCIENCES

Orthotopic Heart Transplantation in the Pig

THERE is only one report of orthotopic heart grafting in the pig¹; the longest survivor lived for 11 d. Heart-lung bypass in this species has proved to be difficult^{2,3}. Our previous studies of organ grafting in pigs made the study of the orthotopically grafted heart interesting to us.

Landrace or large white/landrace littermate pigs, of either sex, weighing 20 to 30 kg were used as donor and recipient. Anaesthesia was with halothane and endotracheal anaesthesia as described for liver allografting⁴. The animals breathed spontaneously until the chest was opened, when the lungs were ventilated mechanically.

With the recipient pig lying on its back, incisions 8 cm long were made in the right iliac fossa and in the neck along each side of the trachea. On the left side an intravenous (i.v.) infusion was set up in the subclavian vein. Arterial pressure, arterial blood gases and pH were monitored by a cannula in the transverse cervical artery and the central venous pressure was measured by a cannula in the internal jugular vein. Heparin (4,000 IU) was given i.v. and a size 18 FG Bardic venous catheter was inserted into the superior vena cava (SVC) through the right external jugular vein. A 3 mm Bentall arterial cannula was inserted into the arch of the aorta through the right common carotid artery. The inferior vena cava (IVC) was cannulated through the right common iliac vein extraperitoneally with a size 20 Bardic venous catheter. The arterial and venous cannulae were connected to the primed lines of the heart-lung bypass machine. Additional tubing was connected from the machine to a low pressure sucker and a left ventricular vent.

Pancuronium (6 mg) was given i.v. and mechanical ventilation was begun. The chest was opened by a median sternotomy. The right pleural cavity was opened, but an attempt was made to leave the left pleural cavity intact, although this was not always possible. A tape was passed around the SVC caudal to the azygos vein; a similar tape was passed around the IVC and both tapes were threaded through rubber tubing to form snares.

For the bypass, the priming volume was 1,300 ml made up of 300 ml of dextran 110 and 1 l of ACD porcine blood less than 24 h old. Added to the priming fluid were 0.5 g potassium chloride, 1 g ampicillin, 4,000 IU heparin, 10 ml sodium bicarbonate (8.4%) and 4 ml calcium chloride (10%). The venous lines passed to two cardiectomy bags from which blood was pumped by a Sarne pump through a heat exchanger and then into a Travenol membrane oxygenator, a filter and then by a second Sarne pump to the arterial line.

The donor pig was anaesthetised by the same technique as the recipient. It lay on its back on the operating table with its left chest rotated uppermost; 3,000 IU of heparin was given i.v. and 6 mg of pancuronium. The lungs were then mechanically ventilated. The abdomen was opened by a midline incision and the abdominal aorta cannulated and connected to an ACD blood collecting bag. Whilst the animal was exsanguinated, the chest was opened on the left side in the fifth interspace, the sternum was divided obliquely and the right chest opened in the fourth space. The pericardium was opened, and the heart removed by first dividing the aorta and pulmonary artery 2 cm from their origins and then dividing the pulmonary veins and venae cavae close to the heart. The heart was inserted into normal saline at 10° C.

For the insertion of the graft, partial bypass was commenced on the recipient. A vent was inserted into the apex of the left ventricle of the donor heart and secured with a catgut purse-string suture. The caval snares were secured and full bypass started. Lung ventilation was reduced to a minimum but not stopped. The aorta and pulmonary artery were clamped and the heart removed leaving most of the atria intact. The donor heart was now removed from saline and the aorta was anastomosed to that of the recipient with a running 4/0 silk suture. Air was expelled from the aorta and the clamp was removed from the recipient aorta and pulmonary artery permitting coronary perfusion. The coronary sinus effluent and blood from the cut hemiazygos vein were returned to the bypass reservoirs via the low pressure sucker. The donor atria and atrial septum were sutured to the recipient atria with running 3/0 mersilene sutures which included the recipient atrial appendages. The pulmonary arterial anastomosis was now completed with a 4/0 running suture and the caval snares were removed. If sinus rhythm had not by now returned spontaneously and there was coarse fibrillation, the heart was defibrillated with a DC shock of 15 joules. If the myocardial tone was poor, 1 ml of 1/10,000 adrenaline was injected into the aorta before defibrillation. The bypass flow was gradually reduced as the donor heart took over the circulation and normal lung ventilation was resumed. Incisions were made in the pericardium

Table 1 Pigs with Orthotopic Heart Grafts

Number	Survival	Cause of death if known
1	2 h	Pulmonary oedema
2	2 h	Haemorrhage
3	4 h	
4	2.5 h	
5	7 h	
6	8 h	
7	12 h	Hyperthermia
8	12 h	
9	12 h	
10	2.5 d	
11	5 d	Rejection
12	5 d	Rejection
13	6 d	Rejection
14	18 d	Rejection
15	4.5 months	Rejection
16	4 months	Alive

to prevent tamponade and a patch of pericardium taken from the donor was sutured to the recipient pericardium to allow for cardiac dilation. The left ventricular vent was clamped and removed after securing a purse-string suture around its site of entry.

When bypass was stopped and blood loss was minimal, the heparin was reversed with protamine sulphate given i.v. and fresh donor blood was infused to maintain a central venous pressure (CVP) of 4 to 10 cm of saline. Dipyridamole (Persantin, 10 mg), deslanoside (Cedilanid, 0.4 mg), calcium gluconate (1 g) and sodium bicarbonate (20 mEq), were infused i.v., and if the blood pressure fell 0.5 mg isoprenaline was given. An Argyle chest drain was inserted by the right ninth interspace. If the left pleural cavity was opened, a similar drain was inserted on the left. The bypass cannulae were removed, the right iliac fossa and neck wounds were closed. The chest was closed with trans-sternal nylon interrupted sutures, continuous nylon to approximate the muscles and subcutaneous tissue and chromic gut for the skin.

The three cannulae on the left side of the neck were left in place for i.v. infusion and monitoring of the central venous pressure, arterial pressure, arterial blood gases and pH. The chest drain was connected to an underwater seal and anaesthesia was stopped when the effect of the pancuronium had worn off. The animal was sedated with Valium (5 mg) given intramuscularly. When breathing movements were judged to be adequate, mechanical ventilation was stopped, and once spontaneous ventilation seemed to be satisfactory the endotracheal tube was removed, the chest drain was joined to a Heimlich chest drain valve and the pig was placed in a warm bed of straw. The following day the chest drain was removed and the animal was given food and drink *ad libitum*. No immunosuppressive therapy was given.

Sixteen pigs survived the operation, but ten died within the early post-operative period. Six animals survived for between 5 d and 4½ months (Table 1). Five of these died with typical stigmata of rejection consisting of round cell infiltration of the myocardium, oedema and vasculitis. One animal was alive and well 4 months after the operation. Animals surviving after 1 week gained weight and seemed to be healthy until the rapid onset of cardiac failure resulting from rejection.

The technique described here was evolved after much trial and error. Long term survival after bypass with bubble oxygenators was not achieved. Their substitution by the Travenol membrane oxygenator seemed to be the chief factor in achieving success with the bypass. Cullum¹ has described the extreme sensitivity of the porcine heart to ischaemia and advocated completing the aortic anastomosis first to revascularise the myocardium as soon as possible. Using this technique the coronary sinus blood tends to obscure the atrial anastomosis and careful suction is required.

At the end of the operation care of the animal is critical, because the pig recovering from anaesthesia is very intolerant

to an endotracheal tube. Once removed, we have not been successful with reintubation. Tachypnoea is marked in the first 24 h and resembles the neonatal respiratory distress syndrome. The loss of surfactant could be a contributory factor. It was hoped that retaining the left pleural cavity intact might reduce respiratory distress but there were long term survivors in animals in whom the left pleural cavity had been opened. Once the animals survived 24 h, recovery was rapid. The pigs resumed normal feeding and seemed to thrive. We were surprised at the long survival in some of our animals. From previous studies by Childe and Morris⁵ and ourselves⁶ on heterotopic porcine heart grafts, rapid rejection was anticipated and this was observed by Cullum with orthotopic porcine cardiac allografts. We have shown that littermate donor/recipient pigs identical for tissue types, and with negative mixed lymphocyte culture reactions, can tolerate renal allografts for many months⁷. As there is a 1 in 4 chance of tissue type identity between two littermates, it is possible that the long survivors described in this communication received hearts from matched donors. This would indicate that the pig reacts to heart or kidney allografts in a similar manner. Further studies are planned on tissue-typed pigs.

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Genetics of Growth in Axenic Medium of the Cellular Slime Mould *Dictyostelium discoideum*

ALTHOUGH *Dictyostelium discoideum* is normally grown on bacteria^{1,2}, it is desirable for most biochemical studies to use strains grown in the absence of bacteria (axenically)³. Isolation of these strains is laborious, however; for this reason we have investigated the genetic basis of growth in axenic medium and have developed a method for inserting the chromosomes bearing the genes for axenic growth into other non-axenic strains. This is made possible by our finding that genes for axenic growth are located on only two of the seven linkage groups and that chromosomes can be readily exchanged between different strains. Using an improved version of the methods employing parasexuality previously reported^{4,5}, we have selected axenic segregants carrying various markers from non-axenic strains.

There are two independently derived strains capable of growth in axenic medium that are commonly used: Ax-2 (ref. 6) (ATCC-24397; derived from strain Ax-1 (ref. 3)) and Ax-3 (ref. 7) (also called A3). Mutants of strain Ax-3 which are

temperature sensitive for growth were isolated using a bromodeoxyuridine selection method (R. H. Kessin and P. C. Newell, unpublished results). Non-axenic temperature sensitive strains M28 and TS12 were obtained from Dr E. R. Katz (Division of Biological Sciences, SUNY, Stony Brook, New York, USA). A temperature sensitive strain (Ax-2g88) of Ax-2 was obtained from Drs E. Gingold and J. M. Ashworth (Department of Biochemistry, Leicester University, England).

Temperature sensitive haploid strains to be fused to form temperature resistant diploids were grown in association with *A. aerogenes* for approximately 48 h at 22° C. Cells were collected from pre-aggregation plates, washed in cold water, and two mutants containing non-identical temperature sensitive mutations were shaken in equal numbers (5×10^6 of each) in 1 ml of water, or in some cases potassium phosphate (20 mM, pH 6.0–7.5) in a Linbro tissue culture dish (FB-16-24-TC). Shaking was for 15–17 h at 22° C, 150 cycles min⁻¹; under these conditions amoebae form 'balls' of aggregated cells². Controls were conducted in which each parent was incubated separately. After incubation the aggregated cells were separated by vortexing, counted with a haemocytometer, and spread with *A. aerogenes* on agar plates at the restrictive temperature (27° C) at 1×10^5 to 3×10^5 amoebae per Petri dish. Temperature resistant diploids were selected at a frequency of about 1 in 10^5 amoebae. Their ploidy was confirmed using genetic markers, spore size, and, in a number of cases, cytological study. Diploids were purified by clonal passage and their spores stored in silica gel at 4° C. It was possible to maintain these diploids by clonal passage; they had a stability similar to that reported by Katz and Sussman⁴.

The method used to form diploids described here considerably facilitates parasexual genetics⁴ since (1) relatively few cells are used; (2) large numbers of crosses can be done at one time in the Linbro tissue culture dish which has twenty-four wells; (3) the method is quick and simple as the cells are not placed on filters; (4) the medium used for fusion can be varied and many parameters can be tested concurrently; (5) it is possible to fuse not only two non-axenic strains (see Katz and Sussman⁴) but also axenic and non-axenic strains and two axenic strains; (6) aggregateless and other morphological mutants can be fused readily using this method.

Growth in axenic medium of strains Ax-2 and Ax-3 was found to be a recessive character since diploids heterozygous for axenic chromosomes (formed by fusion of an axenic and a non-axenic strain) were phenotypically not able to grow in axenic medium. Using this fact it was possible to select haploid axenic strains by placing diploids heterozygous for axenic genes at a low concentration (10^4 – 10^5 ml⁻¹) in axenic medium. After a lag of approximately a week, growth of haploids was usually observed. The haploid axenic strain X2 derived from the diploid of M28 and Ax-3tsg1 was selected in this way (Table 1). Haploid axenic strains were also selected using resistance to recessive drug resistance markers. For example X14 was selected on cycloheximide (500 µg ml⁻¹) from the cycloheximide-sensitive diploid of X9 and NP12 (Table 1).

Diploids homozygous for Ax-3 chromosomes have been isolated. These grow axenically, although subculture in axenic medium results in an ever increasing frequency of haploids until only rare diploid cells remain. This reduction in ploidy from diploid to haploid also occurs when these diploids are grown in association with bacteria without cloning. It has been reported previously that ploidy in the diploid strain H-1 changes from diploid to haploid in bacterial culture in the absence of cloning^{8,9}.

Diploids formed by fusing Ax-2 and Ax-3 haploids grew axenically. Three such independently isolated diploids have been studied; they showed similar growth characteristics in axenic medium to homozygous Ax-3 diploids. Detailed cytological studies on all of these diploids will be reported elsewhere. From these results it seems that the genes involved with growth in axenic medium in the independently isolated strains Ax-2⁶

Table 1 Resegregated Axenic Strains

Parental haploids	Haploid resegregant*	Growth in axenic medium	Growth at 27° C	Brown pigment production	Spore colour	Spore shape	Cycloheximide resistance (500 µg ml ⁻¹)	Acridflavin resistance (100 µg ml ⁻¹)	Colony morphology
M28		—	—	+		Round			
NP2		+	—	—		Elliptical			
	X2	+	—	+		Elliptical			
†X9		—	—	+	White		+		
‡NP12		+	—	—	Yellow		—		
	X14	+	+	+	Yellow		+		
NP29		—	—	+				—	Aberrant aggregation
NP12		+	—	—				+	Normal
	X17	+	+	+				+	Normal

+ Denotes the presence of the characteristic.

* Resegregated strains are called X1 . . . n.

† Strain X9 was selected from the diploid of M28 × NP14.

‡ NP14 and NP12 are acridflavin-resistant strains of TS12 and NP2 respectively; no mutagenesis was used in their isolation. To aid clarity, characteristics which are identical in both parental strains have been omitted.

and Ax-3⁷ may be identical, or at least that the recessive genes involved are the same. We have no evidence yet whether in addition to recessive genes there are dominant genes involved with growth in axenic medium, or indeed how many genes there are. From analysis of segregant classes (to be published elsewhere) it seems, however, that only two linkage groups have genes required for growth in axenic medium. The point to be stressed here is that since ability to grow in axenic medium in strains Ax-2 and Ax-3 is probably not associated with five linkage groups, these linkage groups from non-axenic strains can be readily inserted into axenic strains. By forming appropriate diploids, properties such as cycloheximide resistance, round spore formation, and brown pigment formation have been incorporated into strain Ax-3.

While all of the strains used in these genetic studies are temperature sensitive for growth, it is possible, by using two strains whose temperature sensitive mutations are unlinked, to select temperature resistant (wild type) haploid segregants with various markers. Ax-3 sporulates very poorly at 27° C; this is a serious disadvantage for developmental studies where wild-type and temperature sensitive strains are to be compared. We have selected temperature resistant haploid axenic strains (Ax-3) which show improved sporulation at 27° C. For example, X14 (mentioned previously), Table 1; X17, selected on acridflavin (100 µg ml⁻¹) from the acridflavin sensitive diploid of NP29 and NP12 (Table 1).

Axenic strains grow more slowly on bacteria than non-axenic strains⁸. Some of our temperature sensitive haploid axenics grow very slowly on bacteria, but by selecting haploid axenics with some chromosomes from non-axenics, we have been able to obtain axenic strains with growth rates on bacteria that are much improved (for example, strain X2, Table 1).

While our primary aim is to develop genetic analysis so that factors involved with cell interaction in *D. discoideum* can be studied, strains that are well characterised both genetically and biochemically are necessary. We believe that using the methods discussed here, selection of such strains is facilitated.

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X-ray Studies of the Interaction of CO₂ with Human Deoxyhaemoglobin

In addition to transporting CO₂ indirectly by way of the alkaline Bohr effect, haemoglobin can interact directly with CO₂ to form carbamate residues:



This reaction of CO₂ with haemoglobin is oxygen-linked so that at constant pH more CO₂ is bound to deoxy- than to oxyhaemoglobin, and conversely the oxygen affinity of haemoglobin is decreased in the presence of CO₂ (refs 1 and 2). The studies of Kilmartin and Rossi-Bernardi^{3,4} on horse haemoglobin specifically modified at the N terminal amino groups by reaction with cyanate showed that at constant pH CO₂ has no effect on the oxygen affinity of haemoglobin when all four α-amino groups are carbamylated, but that only part of the oxygen-linked effect is inhibited when the α-amino groups of only the α or only the β chains are blocked. This implies that the N-terminal amino groups are solely responsible for the oxygen-linked CO₂ interactions and that this effect is shared between the α and β chains. More recent experiments have confirmed these results for human haemoglobin⁵.

The X-ray study described attempts to locate the binding of CO₂ to crystalline human deoxyhaemoglobin directly. Because of unpublished evidence (quoted in ref. 2) that concentrated phosphate solutions "affect the reactions of haemoglobin with CO₂", these experiments were carried out with deoxyhaemoglobin crystals⁶ soaked in solutions of 70% 2-methyl-2,4-pentanediol (MPD) to remove the

native high salt mother liquor. Two sets of 3.5 Å diffractometer data were collected, one on crystals soaked in 70% MPD+CO₂/bicarbonate buffer at pH 7.4 (the CO₂ pressure was 1 atm), and another on crystals soaked in 70% MPD+20 mM bis-Tris buffer at pH 7.4. Figure 1 shows the difference electron density, calculated by subtraction of the two sets of data and using the native (high salt) structure factor phases⁷. The expected carbamate group appears as a positive peak, about 3 Å in diameter, immediately adjacent to the α-amino group of the β chains. Next to this positive peak is a large negative peak, which indicates that the native electron density labelled X⁻ in Fig. 1 has been displaced by the newly formed carbamate ion. The same displacement of X⁻ was observed in the X-ray studies of 2,3-diphosphoglycerate (DPG)⁸ and inositol hexaphosphate (IHP; my unpublished work) binding, corroborating the identification of X⁻ as an inorganic anion, probably phosphate or sulphate.

Binding of organic phosphates produced marked changes in the conformation of deoxy β subunits (ref. 8 and my unpublished work) but binding of CO₂ does not seem to affect them. No movements can be detected among the several basic groups in the neighbourhood of the α-amino groups that would indicate the formation of strong carbamate salt bridges. Instead, the carbamate ion seems to be taking the place of the inorganic anion, X⁻, and simply reduces the high concentration of positive charge at the entrance to the central cavity of the haemoglobin tetramer. Similarly, the environment of glutamate 6β—the site of the mutation in sickle cell haemoglobin, haemoglobin S—is unaffected by carbamate formation. Therefore (like DPG^{9,10}) CO₂ should promote sickling at low and medium oxygen saturation levels by stabilising the deoxy conformation, but (unlike DPG^{8,10,11}) CO₂ may not influence the gelation of fully deoxygenated haemoglobin S since under these conditions CO₂ would alter neither the quaternary structure of the tetramer nor the tertiary structure of the β subunits. The CO₂ difference map shows no carbamate formation at the N termini of the α chains. The reasons for this are not clear.

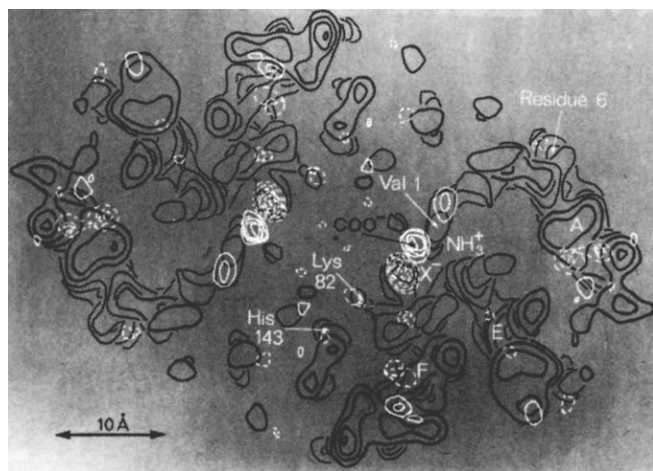


Fig. 1 Fourier map showing the reaction of CO₂ with the N-terminal amino groups of the β chains. Black contours: native human deoxyhaemoglobin electron density map at 3.5 Å resolution (9 sections, -15 to -23 Å). This region includes helices A, E and F and residues Val 1, Glu 6 (the site of the sickle cell mutation), Lys 82 and His 143 of each β chain. These features are related in pairs by the molecular two-fold axis of symmetry. The peak marked X⁻ has been identified as an inorganic anion (see text). The contours are drawn at intervals of 0.15 e Å⁻³ above the zero level. White contours: difference electron density due to CO₂ binding (3 sections, -18, -19 and -20 Å). Positive difference electron density is marked in solid white contours, negative difference electron density is marked in dashed white contours. The first difference contours are drawn at ±0.0091 e Å⁻³, the next contours are at intervals of ±0.0045 e Å⁻³.

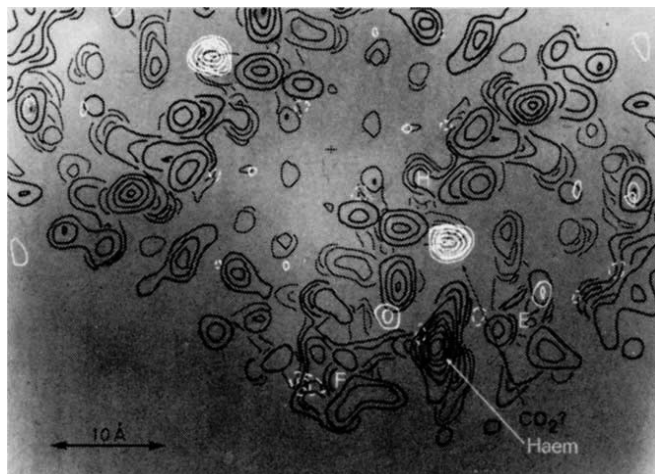


Fig. 2 Fourier map showing the binding of CO₂ to the region of the β-haem group. Black contours: native human deoxyhaemoglobin electron density map at 3.5 Å resolution (7 sections, -8 to -14 Å). This region includes parts of helices E, F and H. White contours: difference electron density due to CO₂ binding (2 sections, -8 and -9 Å) superimposed on the native protein density. Contour levels as in Fig. 1.

There is an unexpected positive difference peak behind the β haem group (Fig. 2). The atomic model of deoxyhaemoglobin shows this strong positive peak to be embedded in the centre of a highly hydrophobic hole. The dimensions of the peak are consistent with the binding of CO₂ or HCO₃⁻, but the hydrophobic nature of the binding site favours the neutral CO₂ molecule. Since physiological CO₂ pressures do not affect the oxygen affinity of cyanate-blocked haemoglobin³⁻⁵, and I have used nearly twenty times the physiological pressure, the binding of CO₂ at this site may be insignificant at these pressures (40 to 50 mm Hg). If it is significant it should not be oxygen-linked; however, it should be remembered that gases do form non-covalent complexes with haemoglobin¹², and that xenon binds to both the α and β chains at specific hydrophobic sites formed by the A-B and G-H corners¹³.

In summary, this X-ray study confirms that CO₂ reacts with the N-terminal amino groups of the β chains to form carbamate ions. It also shows that this reaction displaces an inorganic anion normally bound to the β chain α amino group, but does not cause significant changes in the tertiary structure of the deoxy β subunits. The conditions used in this study of high CO₂ pressure and a low salt MPD environment may, however, have exaggerated a minor effect—the binding of CO₂ in a hydrophobic hole to the rear of the β haem group—and inhibited a major effect—the reaction of CO₂ with the N terminal amino groups of the α chain.

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Cell Shape Regulation and Cell Cycle in Embryonic Lens Cells

EYE lens development in the chicken embryo begins with transformation of the ectoderm destined to become lens from a low cuboidal to a high columnar epithelium. Simultaneously the nuclei pass to the basal poles of the cells. This 'palisading'¹ gives the lens anlage a regular appearance, but individual cells may deviate considerably from the columnar shape, largely because of their replicative behaviour.

All presumptive lens cells are dividing asynchronously at this stage² and show interkinetic nuclear migration³. This phenomenon, first described by Sauer⁴, has been studied extensively in the neural tube, but occurs in many embryonic epithelia. Cells are anchored at their free apical surfaces by intercellular complexes and contract toward this surface for mitosis. Afterwards, during G₁, they elongate again and their nuclei are displaced toward the basal parts of the cells, where DNA synthesis is initiated. At the end of the S phase the nuclei take the reverse route; cells round up again and a new round of mitosis begins. The position and size of the nucleus therefore can be used to classify cells morphologically according to cell cycle phase (our report in preparation). We describe here that the latter is correlated with the surface to volume ratio of the cells and suggest that cell volume regulation may play a role in cell shaping.

White Leghorn chicken embryos, incubated for 48–55 h, were fixed in glutaraldehyde and osmium tetroxide and embedded in Araldite by routine methods. Frontal sections were cut serially at 0.5 μm and stained with toluidine blue. Invaginating rudiments (stages 14 and 15 according to

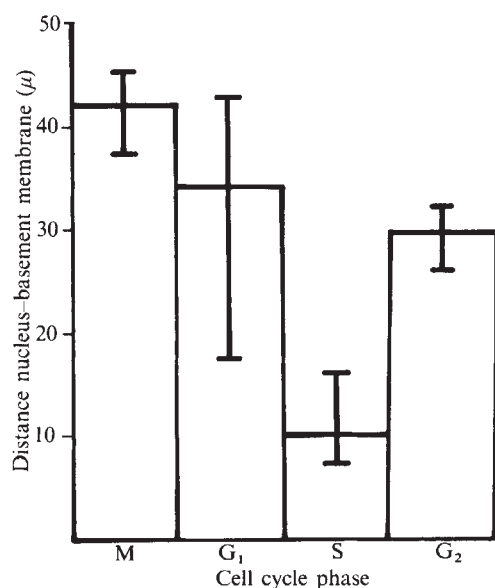


Fig. 1 Histogram indicating the extent of nuclear migration during the replication cycle of cells of the invaginating lens cup. For cells in mitosis the centre of the cell was assumed to correspond with the centre of the nucleus. The vertical bars in this and the following figures indicate the actual range of values; the columns represent the averages of the measurements.

O'Rahilly and Meyer⁵) were selected and from photomicrographs serial tracings were made of lens cells and nuclei. From planimetric measurements we calculated for each cell the distance from the centre of the nucleus to the basement membrane, nuclear volume, cellular surface area and volume, and cell surface to volume ratio.

Figure 1 shows that the nuclei migrate up and down more than 40 μm when the tissue is approximately 50 μm tall. When the nuclear membrane reforms after mitosis the nuclear volume is about 70 μm^3 . It increases to four times as much for the average G₂ cell. In general, nuclei take up more than a third of the cellular volume during interphase (compare Figs 2 and 4).

Measurements of the cellular surfaces show that the average surface area in G₁ is about half that of mitotic cells. The area then increases with the progression of the cell through the cell cycle, but as the cell approaches division and contracts toward the free tissue surface again, the increase slows down and there may even be a slight decrease of surface area (Fig. 3). The changes in cell volume as a function of cell cycle phase show some intriguing differences from the surface changes. Most notably, the value for the G₁ cell is substantially less than the expected half of the mitotic cell volume (Fig. 4). Cell volume then increases, more or less parallel to the surface area curve, but at the end of the cell cycle when the cell approaches division the volume continues to increase instead of diminishing as the surface area does, and the mitotic cell is on average considerably larger than the G₂ cell. As a result, the surface to volume ratio is smallest during mitosis and largest in G₁, with a gradual decrease during S and G₂ (Fig. 5).

We believe that these observations are germane to an understanding of the structural changes of presumptive lens cells in relation to the cell cycle as well as the developmental stage. The decreasing surface to volume ratio in G₂ indicates that the volume increase outpaces the growth of the surface, suggesting the generation of membrane tensile stresses. Entry into mitosis is accompanied by a further substantial

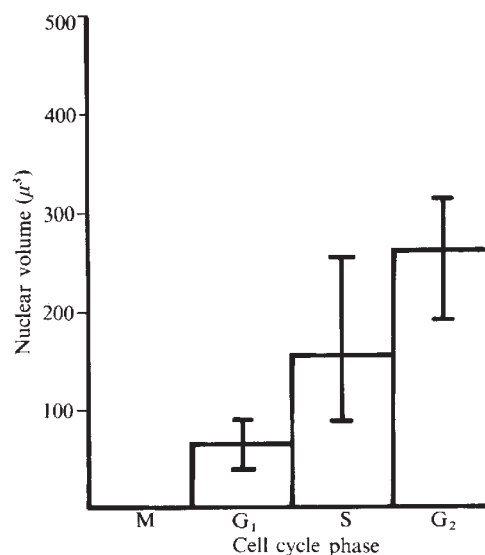


Fig. 2 Cell nuclei become larger with the progression of the cell cycle. Obviously, during division the nuclear membrane has become dissociated and the nuclear volume cannot be measured. Nuclear volumes were determined in the same way as cellular volumes (Fig. 4).

increase in cell volume and a slight decrease in surface area. A sphere has the smallest surface to volume ratio of all possible configurations of a given volume and, moreover, this ratio diminishes with increasing size. Geometry makes it tempting, therefore, to postulate that volume increase

forces the cell to round up in preparation for mitosis. The very rapid change in volume may be due to an influx of fluid from the cellular environment, an explanation compatible with the low density of the mitotic cell, as judged from staining characteristics. These interpretations agree

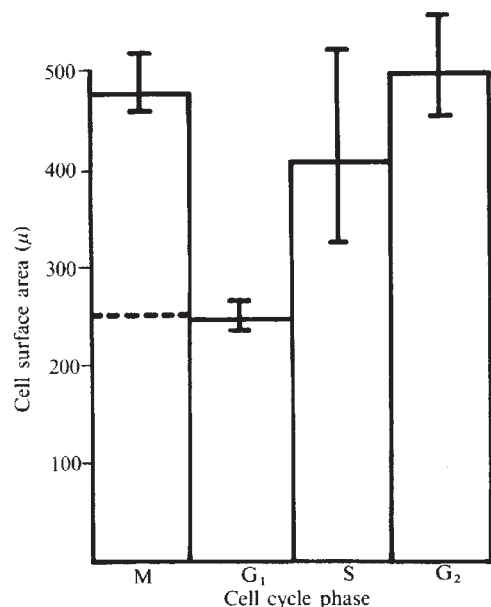


Fig. 3 The average cell surface as a function of cell cycle phase. The surface of each cell was calculated by summing all measured outlines of the cell in serial photomicrographs and taking into account the magnification factor and the thickness of the sections. Electron microscopy indicates that the surface of lens cells at this stage is very regular. Therefore, the outline of the cell at the light microscopic level does in fact represent its surface. Note that the average surface area of the G₁ cell is about the same as half the surface of the mitotic cell (broken line in first column). The surface area becomes larger with the progression of the cell cycle, but appears to diminish somewhat at the end, with the transition from G₂ into M.

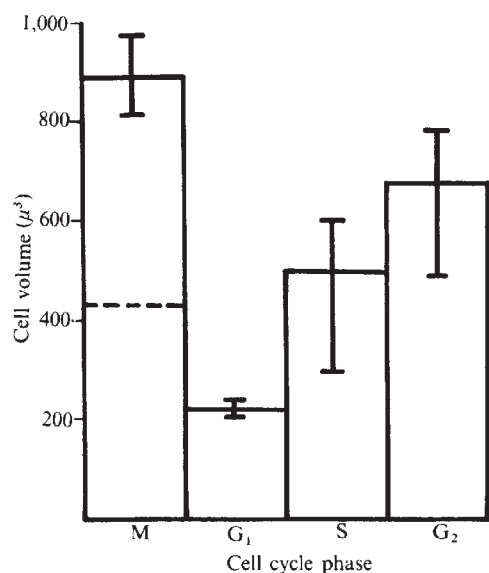


Fig. 4 Cell volume in relation to the cell cycle. The volume was calculated by planimetrically determining the surface of each section through a cell from serial photomicrographs, summing all the areas, and taking into account the magnification and the section thickness. In striking contrast with the surface area changes (Fig. 3) the G₁ cell volume is much smaller than that of the halved mitotic cell. The volume increases continuously over the cell cycle, and the dividing cell is clearly larger than the G₂ cell.

with earlier results: it is well known that cleavage of sea urchin eggs is accompanied by increase of tension in the cell membrane⁶, and L cells in culture show appreciable increases in volume and decreases in surface to volume ratio when division begins⁷.

With the transition from mitosis to G₁, the opposite occurs. The division of a sphere into two smaller spheres requires more surface area, relative to the same total volume. This requirement increases when the cell elongates in G₁. Theoretically, the cell can only attain this change in structure by increasing its surface or decreasing its volume. Apparently the latter is favoured in this system, the average G₁ cell volume being considerably less than the expected half of the mitotic cell volume. Because G₁ cells stain very intensely we believe that loss of cytoplasmic fluid may be involved. Whether active transport phenomena are responsible remains to be determined.

Lens placode formation is characterised by cellular elongation and it seemed possible that the extent of the latter is regulated by controlling the volume, apparently lost by the post-mitotic cell. Preliminary work indicates that this is not the case. Throughout early lens development the changes in cell volume and surface in relation to the cell cycle are comparable with those described here for the invaginating rudiment. Thus, we hypothesise that the high surface to volume ratio of the G₁ cells allows it to assume various shapes, including the columnar one. Conversely,

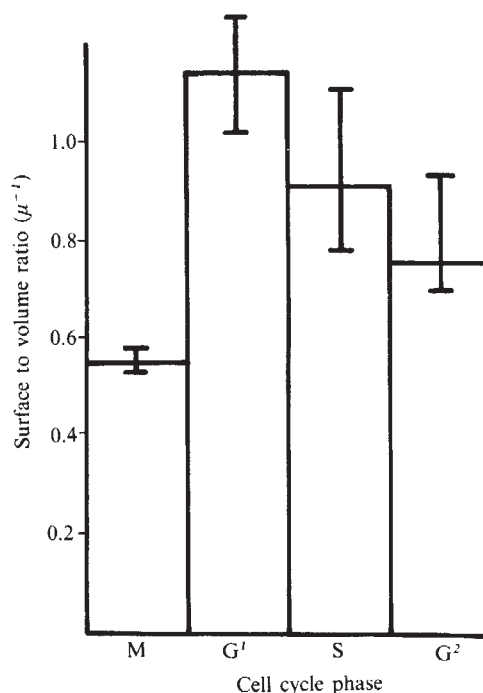


Fig. 5 The surface to volume ratio decreases from high values for G₁ to the lowest ones for mitotic cells, indicative of a progressive limitation of the theoretical freedom that the cell has to assume various morphological configurations. Geometric laws dictate that a structure with a high surface to volume ratio can have any number of shapes, while a low ratio has to be associated with a spheroid form.

the diminishing of the ratio in G₂ limits this freedom and pushes the cell towards a spherical form. Cell volume decrease in G₁ permits the cell to elongate, but it does not instruct it to do so; other forces must cause the elongated shape to be favoured⁸.

Our observations indicate that regulation of cell volume may well delineate the options open to a cell in the determination of its shape. Except for Cone's work⁷, this aspect has received little attention. If the volume adjustments

found here turn out to involve active transport mechanisms, as we suspect, the associated osmotic changes may well be important in the control of mitosis⁷ and DNA synthesis⁹. Our methodology should be applicable to other systems to facilitate classification of cells according to cell cycle phase without reference to synchronisation and (or) isolation of cell populations.

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Precursor 16S RNA in Active 30S Ribosomes

BIOGENESIS of bacterial ribosomes proceeds through the formation of precursor particles¹⁻⁴, consisting of an incomplete set of ribosomal protein⁵ and of RNA molecules that are longer and less methylated than mature RNA^{4,6,7}. RNA

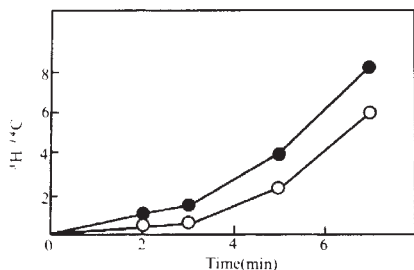


Fig. 1 ³H-uracil uptake in m16S RNA and 30S particles. A culture of *Escherichia coli* D10 in medium MS9 (ref. 9) supplemented with 0.5% casa-amino acids (doubling time 32 min) was labelled with ¹⁴C-uracil (0.1 μC ml⁻¹). After two generations, ³H-uracil was added (5 μC ml⁻¹). At indicated times, samples were rapidly chilled and lysed as previously described¹⁰. A portion of each lysate was centrifuged through a 10% to 30% sucrose gradient in 10⁻⁴ M MgCl₂, 10⁻² M Tris-HCl, pH 7.8, at 40,000 r.p.m. for 8 h to display 30S ribosomes and precursor particles. The peak fraction of the 30S region of each gradient was diluted with three volumes of 10⁻⁴ M MgCl₂ and centrifuged through a second sucrose gradient to resolve 30S ribosomes from precursor particles completely. The ratios of ³H to ¹⁴C counts in 30S ribosomes are plotted against labelling time (●—●). Another portion of each lysate was treated with phenol and sodium dodecyl sulphate to extract the RNA. After ethanol precipitation, the RNA was electrophoresed for 6 h at 5 mA per tube on 3.6% polyacrylamide gels prepared as previously described¹⁰. The ³H to ¹⁴C ratios in m16S RNA were multiplied by a factor 1.5 and then plotted against time (○—○). This correction was necessary to obtain comparison with 30S ribosome ratios, since different conditions were used for counting sucrose gradient and gel fractions. ³H and ¹⁴C counting efficiency was controlled for each experiment by including an extra gradient with 30S ribosomes uniformly labelled with ³H and ¹⁴C uracil, and an extra gel with 16S RNA extracted from the same 30S particles.

maturation is commonly considered to be a part of the ribosome maturation process. Here we report that newly formed 30S ribosomes can enter polyribosomes while they still contain precursor RNA. Maturation of ribosomal RNA might be completed not before, but after, ribosomes have interacted with messenger RNA.

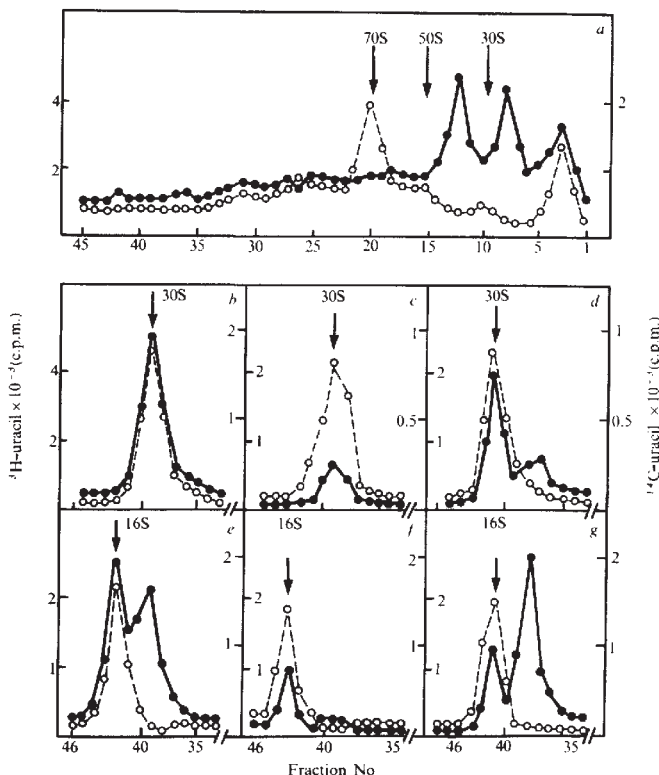


Fig. 2 Newly formed RNA in native and derived 30S subunits. *a*, A lysate of cells labelled with ¹⁴C-uracil for two generations and with ³H-uracil for 3 min, as in Fig. 1, was centrifuged on a sucrose gradient in 10⁻² M MgCl₂, 10⁻² M Tris-HCl, pH 7.8, for 2 h at 40,000 r.p.m. to display free and messenger-bound ribosomes. Fractions 25–42 were pooled and dialysed at 2° C against 10⁻⁴ M MgCl₂, 10⁻² M Tris-HCl, pH 7.8. Ribosomal subunits were collected by centrifugation and resuspended in 0.5 ml of the dialysis buffer. This sample, as well as fractions 20 and 10 of the original gradient, were centrifuged on three parallel gradients in 10⁻⁴ M MgCl₂ for 8 h at 40,000 r.p.m. to display 30S subunits. *b*, 30S derived from polyribosomes (fractions 25–42 of *a*); *c*, 30S derived from 70S (fraction 20); *d*, native 30S (from fraction 10). From the peak fraction of each of these three gradients RNA was extracted and analysed by gel electrophoresis as in Fig. 1. *e*, RNA from polyribosomal 30S; *f*, RNA from 30S derived from 70S; *g*, RNA from native 30S. The top of gradients and of gels is at the right. ●, ³H-uracil c.p.m.; ○, ¹⁴C-uracil c.p.m.

As we have briefly noted elsewhere⁸, conversion of 17S and 16.5S RNA (p16S) into mature 16S RNA (m16S) does not occur at the same time as that of precursor particles into 30S ribosomes. In the experiment summarised in Fig. 1, we have followed the entry of ³H-uracil into m16S RNA and 30S particles during continuous labelling. A delay of 1 to 2 min in m16S RNA labelling was found by comparison with 30S particles.

This suggested that newly formed RNA is incorporated in particles sedimenting as 30S while it is still immature. These particles might be precursors not resolved from mature ribosomes on sucrose gradient, like those recently described by Lindahl¹¹. When we examined the distribution of newly synthesised RNA in various ribosomal fractions we found, however, that p16S was present not only in native 30S ribosomes¹², but also in 30S subunits derived from polyribosomes (Fig. 2). The ratio between mature and immature RNA was

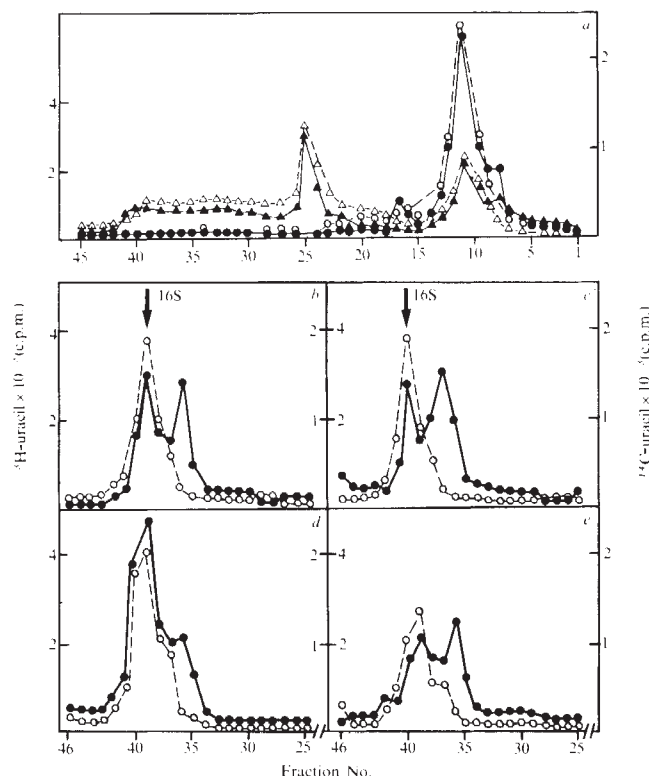


Fig. 3 Formation *in vitro* of polyribosomes from native subunits and T4 RNA. Native 30S particles were isolated by sucrose gradient from a lysate of cells prelabelled with ^{14}C -uracil and labelled with ^3H -uracil for 3 min. The labelled particles were mixed with a crude extract (S30) prepared from unlabelled cells, and incubated in conditions of protein synthesis with or without T4 RNA¹⁰. S30 had been preincubated at 37° C for 10 min. After 2 min of incubation chloramphenicol was added (200 $\mu\text{g ml}^{-1}$) and the mixture was centrifuged on a sucrose gradient in 8 mM MgCl_2 , 10 mM Tris-HCl, pH 7.8, for 2 h at 40,000 r.p.m. *a*, Gradient distribution of 30S incubated for 2 min with ($\blacktriangle$, ^3H ; $\triangle$, ^{14}C) and without ($\bullet$, ^3H ; $\circ$, ^{14}C) T4 RNA. RNA was extracted from fractions 26–40 and from fraction 10 of the first of the two gradients. *b* and *c* show the gel analysis of these two preparations of RNA, in that order. The top of gels is at the right. The same analysis was made on 30S ribosomes incubated with T4 RNA for 4 min. The gradient is not shown. In *d* and *e*, gel patterns are given for RNA extracted from polyribosomes and free 30S.

different in the two fractions. After 3 min of labelling, native 30S particles contained most of their ^3H label as p16S RNA; 30S derived from polyribosomes contained half of their ^3H label as p16S and half as m16S RNA. 30S subunits derived from 70S monosomes contained only 16S RNA. Later the portion of ^3H label increased in all fractions.

These results indicate that immature RNA can be incorporated in particles that not only sediment as 30S, but can also enter polyribosomes. The relationship between ribosomal subunits found free in a cell extract and those in polyribosomes and 70S monosomes is not yet completely clear¹³. The ratio of immature to mature RNA in these fractions is, however, consistent with the hypothesis that newly formed 30S ribosomes enter polyribosomes while they still carry p16S RNA and are released after the RNA is converted to m16S. Maturation of the RNA would then occur in polyribosomes.

This interpretation is supported by *in vitro* experiments in which native 30S particles were tested for their ability to respond to natural messenger RNA. Labelled 30S ribosomes were added to a cell crude extract and incubated in conditions of protein synthesis (Fig. 3). In the presence of T4 phage specific RNA, newly formed ^3H -labelled 30S particles formed 70S and polyribosomes with the same efficiency as the pre-existing ^{14}C -labelled 30S. After the first 2 min of incubation

in vitro, the ^3H label that had entered polyribosomes was distributed between m16S and p16S RNA in the same proportion as in free 30S particles. After 4 min, the ^3H fraction in m16S was higher in polyribosomes than in free subunits. In the absence of T4 RNA, no formation of polyribosomes and no conversion of p16S into m16S RNA were observed under the same conditions. These data confirm that 30S particles containing p16S RNA can enter polyribosomes, and once again suggest that maturation of the RNA occurs preferentially in polyribosomes.

We were unable to test whether the particles containing p16S RNA are fully competent in protein synthesis because we could not separate them from those containing m16S RNA. For the same reason, we could not determine their protein content. Furthermore p16S RNA was identified by gel electrophoresis, but was not characterised further. Therefore the possibility that RNA maturation plays a role in the structural or functional maturation of ribosomes cannot be excluded.

The data presented here, however, suggest an additional possibility, namely that complete RNA maturation may not be required to make a ribosome functional. On the contrary, the functioning of a ribosome may be necessary for its RNA to mature. A conformational change of the particle, for example, may be required to expose the RNA to a nuclease attack. In this case splitting of the extra nucleotide sequences from the immature RNA molecules would occur at a rate determined by the chance which newly formed ribosomes had of entering polyribosomes, and therefore could serve to signal how many new ribosomes are needed.

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Antibody-dependent Killing of *Cryptococcus neoformans* by Human Peripheral Blood Mononuclear Cells

CRYPTOCOCCOSIS is a systemic fungus infection caused by the encapsulated yeast-like fungus, *Cryptococcus neoformans*. It has been suggested that alterations in cellular host defence mechanisms increase susceptibility to infection with this organism^{1,2}. *In vitro* studies suggest that human peripheral blood neutrophils and monocytes can ingest and kill crypto-

cocci^{3,4}. There is, however, a wide range of killing in cells from different normal subjects (21–89%), and no apparent defect in cells from patients with cryptococcosis³. This makes it unlikely that peripheral blood neutrophils or monocytes play a decisive role in determining susceptibility to cryptococcosis in individual patients. In other *in vitro* studies, activated macrophages derived from human peripheral blood monocytes not only failed to kill cryptococci, but provided a preferentially favourable medium for intracellular growth of the fungus⁵. These studies, as well as the known histopathology of cryptococcal lesions, suggest that many of the organisms, particularly large capsule forms, are extracellular and so not subject to the intracellular killing mechanisms of phagocytes. Anti-cryptococcal antibody, with or without complement, does not kill cryptococci in the absence of leukocytes³. I therefore investigated the possible existence of a non-phagocytic cellular killing mechanism.

Mononuclear cells were separated from heparinised human peripheral blood from normal donors as outlined by Böyum⁶. Final preparations consisted of 20–38% monocytes (by neutral red ingestion), plus 62–80% lymphoid cells of other types. A small capsule isolate of *Cryptococcus neoformans* was cultured 72 h on Sabouraud's agar and washed as previously described³. Anti-cryptococcal antibody was raised in rabbits and standardised by slide agglutination as outlined by Wilson *et al.*⁷. The agglutination titre of serum used in these studies was 1:512. Control serum was obtained from the same rabbit before development of antibody.

If phagocytosis is blocked, it seems that mononuclear cells can readily kill cryptococci only if anti-cryptococcal antibody is present. Maximum killing was noted after 24 h incubation (Table 1). At 4 h, 45.5% of the original inoculum survived. Beyond 24 h, results were difficult to interpret because of variable growth of remaining fungi. Microscopic examination confirmed that clumping of organisms could not explain the apparent killing which was observed. Similarly, little or no phagocytosis of organisms was noted, as expected in a system nearly free of serum. But the high ratio of leukocytes to fungi in this system allowed the possibility that a small amount of phagocytosis might be missed, yet still result in significant killing. Therefore, incubations were performed with 10 µg ml⁻¹ cytochalasin B, which completely inhibited phagocytosis of cryptococci. Others have noted that this concentration does not block antibody-dependent cell-mediated cytotoxicity⁸, and only partially blocks cytotoxicity by immune spleen cells if cells are mixed to facilitate contact⁹. In three paired experiments, killing as reflected by percentage survival of the original inoculum was not significantly changed at 24 h in incubations containing cytochalasin B (mean ± s.e. of the mean survival 5.3 ± 5.3%, changed to 9.2 ± 6.9% with cytochalasin B).

Further dilution of antibody to 1:20,000 did not reduce killing (6.6% survival), but at 1:200,000 dilution, 73.3% of the original inoculum survived at 24 h. The optimum ratio was 50–100 leukocytes per cryptococcus. At a 20:1 ratio, 72.8% of the original inoculum survived, increasing to 88.0% at a 5:1 ratio. No killing was observed at 1:1 ratio (100% survival).

Preliminary studies indicated that human antibody can act in this system. Serum obtained from a patient with cryptococcosis was found to contain anti-cryptococcal antibody by an indirect fluorescent antibody technique¹⁰. When incubated in a 10% final concentration with mononuclear cells from two normal donors plus 10 µg ml⁻¹ cytochalasin B to block phagocytosis, only 7.3% and 28.1% of the original cryptococcal inoculum survived at 24 h. In another experiment, normal serum was used which had no detectable anti-cryptococcal antibody by the indirect fluorescent antibody technique, but did have antibody present by a more sensitive absorption technique¹¹. The IgG fraction from this serum was separated by the DEAE batch technique of Stanworth¹². When 11 mg ml⁻¹ was added to incubations, only 29.2% of the original cryptococcal inoculum survived at 24 h.

Table 1 Effect of Anti-cryptococcal Antibody on Survival of Cryptococci cultured with and without Mononuclear Cells

Leukocytes present *	Antibody present †	Survival of original cryptococcal inoculum (% at 24 h ‡)
Yes	Yes	(6.8 ± 3.2)
Yes	No	(111.3 ± 7.6)
No	Yes	(158.0 ± 43.0)
No	No	(186.8 ± 49.0)

* 50–100 leukocytes : 1 cryptococcus.

† 1:2,000 dilution of hyperimmune serum or serum from the same rabbit obtained before development of antibody.

‡ Mean ± s.e. of the mean of experiments using leukocytes from nine donors. Original inoculum represented as 100%.

Triplicate 35 mm plastic dishes each contained approximately 5 × 10⁴ cryptococci quantified by plate counts, with or without varying ratios of leukocytes in 1 ml of medium. Media consisted of heated (56° C for 30 min) rabbit serum diluted 1:2,000 or greater with Eagle's minimal essential medium enriched as outlined elsewhere²⁰. Dishes were incubated at 37° C in 5% CO₂ with continuous mixing on a rocker platform for 24 h. Leukocytes were lysed and clumps dispersed using Triton X-100 in saline (1:1,000 final dilution when added to culture dishes). Dishes were scraped with a rubber policeman and washed four times with Triton X-100 into a test tube which was vigorously mixed. This treatment did not decrease viability of cryptococci. Further tube dilutions were performed in distilled water. Live organisms were quantified by plate counts and the number compared with the original inoculum to measure any growth or killing which occurred during the period of incubation.

Separated mononuclear cells suspended in 10 ml of medium were incubated in 100 × 20 ml plastic dishes on rocking platforms at 37° C for 30 min in 5% CO₂. Non-adherent cells were removed and the procedure was repeated twice. When these non-adherent cells were used in incubations, killing was reduced 60% compared with incubations where adherent cells were not removed. This suggests that the killer cells in this system have some capacity to adhere to surfaces. If this system is analogous to antibody-dependent cell-mediated lysis of erythrocytes or other target cells as described by several workers^{13–17}, my results are consistent with those reported by Van Boxel using mouse spleen effector cells¹⁷. Studies are in progress to identify the specific effector cell which can kill cryptococci. Because of the heterogeneous nature of the mononuclear cell population used in this system, the possible roles of monocyte-like cells¹⁸, B lymphocytes¹⁷, or other cells is still uncertain.

In any case, it seems that mononuclear cells in the presence of specific antibody can kill a microorganism, *C. neoformans*, by a non-phagocytic mechanism. The relevance of these observations to *in vivo* conditions remains to be established. It may, however, be noteworthy that the presence of anti-cryptococcal antibody in the serum of patients with cryptococcosis has been correlated with eventual cure¹⁰. Antibody-dependent cell-mediated killing as well as other cellular non-phagocytic killing mechanisms¹⁹ may well be important in dealing with microorganisms which are not readily ingested by phagocytic cells.

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Mechanism of Action of Dental Electro-anaesthesia

NEWMAN¹ has proposed that the analgesia produced by passing a small electric current from the dental drill (positive) to a hand electrode (negative) is due to anodal blocking of nerve fibres within the tooth. Fields *et al.*² feel that the likely mechanism is electrocoagulation of odontoblasts in the dentinal tubules under the site of drilling. The difference is clinically important, for anodal blocking is a harmless reversible process whereas the destruction of odontoblasts leaves permanent dead tracts in the dentin under the site of application of the current. These two mechanisms can be separated by applying stimulation and electro-anaesthetic current to different sites on the same tooth, since the local mechanism (electrocoagulation) would have no effect in blocking a stimulus applied elsewhere.

Ten cats were prepared with chronic amalgam implants on the upper canine teeth^{3,4}. Eight of these had two-electrode implants; two were prepared with three-electrode implants. The stimulating, anaesthetising, and recording arrangements were as shown in Fig. 1. Current was applied from an implant close to the apex of the pulp chamber, with a return lead from a stainless steel screw embedded in the skull, simulating normal dental electro-anaesthetic practice. Test stimuli were applied from another tooth implant to the skull (Fig. 1A), or between two other tooth implants (Fig. 1B). The jaw opening reflex^{3,4}, monitored electromyographically, was used as the index of pulp excitation. The cats were tested repeatedly at intervals over a period of 3 to 4 months. All were awake; the test stimulus used does not induce overt distress or escape behaviour in the cat provided the pulse is brief, mild and not too frequent^{4,5}.

Typical results are shown in Fig. 2. Anodal current reduced or blocked the reflex response; cathodal current increased it. The smallest effective current was 20 μ A, and typically 40 to 60 μ A was required for a full block. The blocking tended to be cumulative, in the sense that successive blocks required less current. There was, however, complete recovery between sessions.

In a few trials, much larger currents (500 to 2,000 μ A) were used for comparison with the practice of ionic desensitisation^{6,7}, which is known to produce local damage to odontoblasts. On cessation of this blocking current, the cats tested were visibly distressed and drank copiously; there were no obvious permanent after effects.

The currents found adequate to block the jaw opening reflex in this study are comparable in magnitude with those

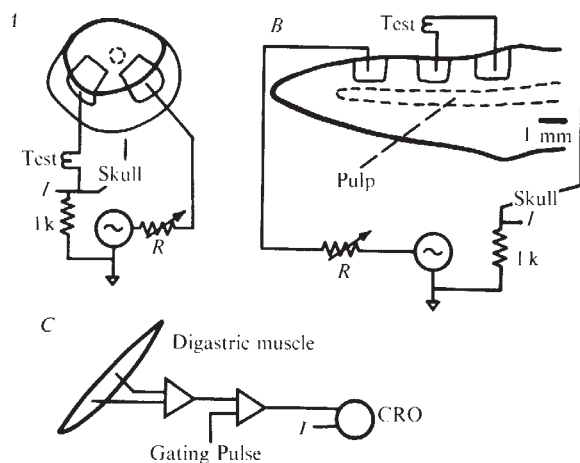


Fig. 1 Three-terminal (A, eight cats) and four-terminal (B, two cats) arrangements used for testing anodal blocking of pulp nerves. The test pulse was 0.2 ms long, 200 to 500 μ A in amplitude, derived from an r.f.-isolated bipolar source which had an internal resistance of 100 k Ω . In arrangement A the polarising and test currents both passed through the tooth root to the skull; in B the test pulse was confined within the tooth. Point I was connected to the lower beam of the oscilloscope to record current passed through the polarising electrode. The signal generator was operated at constant voltage (centre zero, range ± 10 V) and the polarising current was varied by changing R within the range 0.1 to 1.0 M Ω . The digastric EMG was monitored as shown in C. Hook electrodes, of platinum or nichrome wire, 1 cm long and 0.25 mm diameter, were chronically implanted about 1 cm apart in each digastric muscle. Leads from a skull plug were run to a differential preamplifier of gain 100, bandwidth 80 Hz to 10 KHz, input impedance 10 M Ω . The output from this amplifier went to a gated amplifier which displayed, on the upper beam of the oscilloscope, the segment of EMG waveform occurring 4 to 13 ms after each test stimulus (see Fig. 2).

effective in clinical electro-anaesthesia^{1,2,8}. The site of testing is separated from the site of injection of the polarising current, so the block of response cannot be due to an inactivation of receptors in the dentin under the polarising electrode. The reversible blocking seen is consistent with the known properties of anodal blocks on other nerves⁹. Thus, it seems that anodal polarisation of pulp nerves may be a significant mechanism in the clinical relief of tooth pain. These findings do not exclude the possibility that local damage to odontoblasts may also play a role. Fears of clinically significant damage produced by currents of 60 μ A or less are, however, probably unwarranted, since much larger currents have been used routinely for dentin

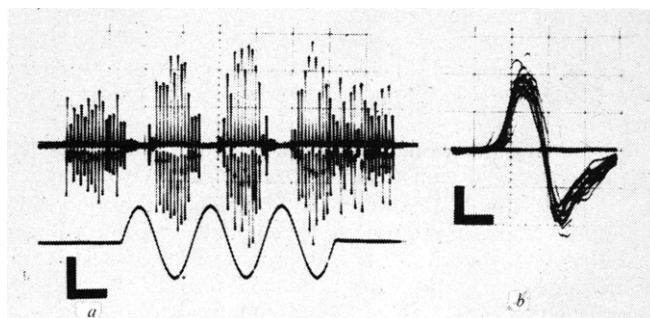


Fig. 2 a, Variation of digastric EMG amplitude with polarising current. Each vertical line on the upper trace is a 9 ms segment beginning 4 ms after a test stimulus to the tooth. Test stimuli are applied at a rate of 2 s⁻¹. Calibration: upper trace, 2 mV; lower trace, 20 mV (equivalent to 20 μ A); time, 5 s. Lower trace shows the polarising current applied; tooth positive (anodal) is up. b, Detail of EMG waveform. Ten sweeps with stimulus, five without. Calibration: 2 mV; time, 2 ms. Only the gated portion of the response is shown.

desensitisation for many years^{6,7}.

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Variation with Sex of Irradiation-induced Chromosome Damage in Somatic Cells of *Drosophila melanogaster*

IN *Drosophila melanogaster*, much information has been collected on chromosome mutations analysed by genetic methods, but there has so far been no cytological analysis of the chromosome mutations induced in somatic cells. By irradiating cells of *D. melanogaster*, in which we knew that somatic pairing was present, we have also wished to extend our previous studies¹, carried out on endoreduplicated Chinese hamster cells, to verify the influence of chromosome pairing on the type and frequency of induced chromosome aberrations.

Third instar larvae of two stocks (Oregon R and *b cn vg bw*) were irradiated with 312.5, 625 and 1,250 R of X rays (180 kV, 6 mA, 3 mm Al and 125 R min⁻¹). At various times after irradiation, samples were prepared by squashing larval ganglia in acetic orcein after fixation for 3 min in a 1 : 1 mixture of methyl alcohol and acetic acid. Before fixation, the ganglia were

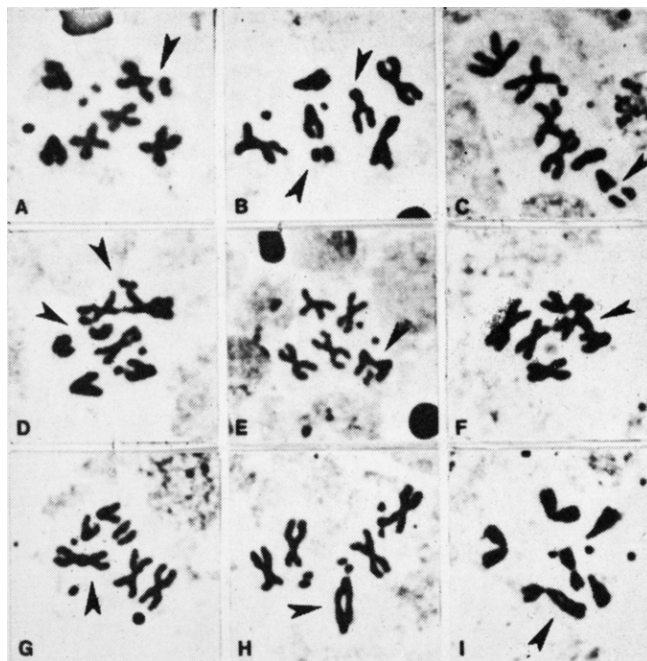


Fig. 1 Induced chromatid and chromosome aberrations in larval ganglion cells of *D. melanogaster*. A, (♀) Terminal deletion of an autosome; B, (♀) isochromatid deletion of an autosome; C, (♂) isochromatid deletion of an X chromosome; D, (♀) asymmetrical exchange between autosomes plus isochromatid deletion of an autosome; E, (♀) asymmetrical exchange between X chromosomes; F, (♀) symmetrical exchange between autosome and X chromosome; G, (♀) dicentric between autosomes with associated fragment; H, (♀) dicentric between X chromosomes with associated fragments; I, (♀) translocation between autosomes.

kept for 2 h in a 0.7% aqueous solution of NaCl containing 10⁻⁴ M colchicine.

Figure 1 shows the main types of chromosome and chromatid aberration that we could score in this material. The genome of *D. melanogaster* is suited to this type of analysis, as the

Table 1 Frequencies of Chromatid and Chromosome Aberrations in Larval Ganglion Cells of *D. melanogaster* after Treatment with X Rays

Stock	Dose (R)	Fixation time post-irradiation (h)	Sex	No. of cells with aberrations	Chromatid deletions	Isochromatid deletions and chromosome deletions†	Chromatid exchanges	Dicentrics	Scorable translocations and inversions	Chromatid gaps	Isochromatid gaps
Control	0	—	♀ ♀	5	1	4	—	—	—	—	2
			♂ ♂	2	2	—	—	—	—	1	—
	312.5	4	♀ ♀	178	18	181	7	—	—	6	4
			♂ ♂	123*	13	111*	2	—	—	5	3
	625.0	4	♀ ♀	316	39	326	20	1	—	6	8
			♂ ♂	225*	32	205*	10	3	—	5	9
Oregon R	2	2	♀ ♀	540	219	338	130	—	—	31	17
			♂ ♂	448*	148*	306	72*	—	—	28	12
	1,250.0	4	♀ ♀	505	115	477	54	16	—	16	18
			♂ ♂	362*	72*	357*	16*	8	—	11	10
		8	♀ ♀	436	19	394	4	125	30	5	4
			♂ ♂	314*	25	261*	3	77*	12*	3	4
		10	♀ ♀	435	10	296	—	238	36	4	2
			♂ ♂	294*	6	233*	3	110*	22	3	2
<i>b cn vg bw</i>	625.0	4	♀ ♀	409	94	376	17	10	1	13	11
			♂ ♂	321*	60*	285*	9	8	—	14	10

* Significant in χ^2 test with $P < 0.01$.

† An isochromatid deletion can result both from the replication of a chromosome breakage (chromosome deletion) and from an isochromatid breakage. Consequently this type of aberration is present at both early and late fixation times post-irradiation. 1,000 metaphases of at least ten larvae scored for each point.

aberrations are clear and the number of chromosomes per cell is small and two pairs are easily distinguished from the others. In the colchicised metaphases, the somatic pairing is lost; however, the pairing is clearly present up to late prophase.

Table 1 shows the data obtained in one series of experiments. The experiments were carried out mainly on the Oregon stock and then on the *b cn vg bw* stock for confirmation. The larvae of the Oregon stock irradiated with 1,250 R were killed at various times after irradiation to obtain data on the different stages of the cell cycle: in this way it was possible to study chromatid and chromosome aberrations.

The main result that emerges from the data of Table 1 is that, in the females of both the stocks at all three doses of irradiation, there is a greater frequency of chromosome or chromatid aberrations of every type.

In the present state of our studies it is difficult to determine the cause of this difference: a difference in the efficiency of repair both of premutational damage and of the broken chromosomes might be involved^{2,3}. In this respect it is natural to think of the possible presence in the females, as opposed to the males, of the enzymes involved in crossing-over, and of the possibility that, for the formation of a chromosome aberration, some kind of rectification of lesions in DNA is necessary^{4,5}.

Another fact worth emphasising is the almost total absence in our results of intra-exchanges, either at chromatid or at chromosome level. We maintain that this is one of the effects associated with the chromosome pairing.

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Radiochemical Purification of Bacteriophage λ Integrase

BACTERIOPHAGE λ has evolved a highly specific mechanism by which it integrates its DNA into the *E. coli* genome. The product of the lambda gene *int*, which I call integrase, mediates the insertion of the phage DNA at specific attachment sites on both the phage and host genomes¹⁻³. A non-enzymatic method by which integrase can be identified in infected cells has been described previously^{4,5}. It depends on the observation that in cells which have been heavily irradiated with ultraviolet light before infection with phage λ , synthesis of host proteins and most phage proteins is markedly depressed⁶. Under these conditions a high percentage of labelled amino acids is incorporated into phage-specific proteins including integrase. Using a differential labelling technique, integrase is identified by means of sodium dodecyl sulphate (SDS) gel electrophoresis as the only protein species eliminated by a nonsense (chain-terminating) mutation in the *int* gene.

I describe here the purification of integrase, monitored by electrophoretic assay, to radiochemical homogeneity. In an independent study, Nash⁷ has used a similar method to obtain radiochemically pure integrase, which he calls Int protein.

Figure 1a illustrates electrophoretic identification of

labelled proteins synthesised in a heavily irradiated host by either *int*⁺ phage or *intam* (*int*29) phage. One major peak at molecular weight 44,000 is synthesised only by the *int*⁺ phage and is thus concluded to be integrase.

The interpretation that the peak at 44,000 molecular weight is integrase is corroborated by the following experiments. (1) I isolated two additional *int* amber mutants, *int*405 and *int*920, and these mutations also eliminate the peak at molecular weight 44,000. *Int*405 is different from *int*29 and *int*920 since it is suppressed only by *su*_T⁺ hosts whereas *int*29 and *int*920 are both suppressed by *su*_T⁺ hosts. (2) The protein peak at molecular weight 44,000 is found if the infection with *intam* phages is carried out in a *su*⁺ host. (3) The *bio*16-A deletion spans a region of DNA, from the attachment site to within the *int* gene⁸, which would code for a polypeptide of molecular weight approximately 22,500 (ref. 9). I have shown that the *bio*16-A deletion eliminates the peak at molecular weight 44,000, thus ruling out the possibility that this peak is coded by a gene adjacent to *int* but is eliminated by *int* amber mutations because of polarity. (4) The peak at molecular weight 44,000 is not affected by the non-suppressible mutation *int*6 (ref. 10) or by the temperature-sensitive mutation *int*1 (ref. 11). This experiment indicates that this peak contains the protein defined by the *int* amber mutations rather than a protein under *int* control or a protein controlling integrase production.

We have shown previously that in crude extracts integrase is associated with large, fast-sedimenting particles⁹. Treatment at high ionic strength dissociates integrase from these particles and leaves it in a slower sedimenting form. This observation forms the basis for a purification procedure. Large integrase particles are collected by centrifugation in low salt and then resedimented in 0.66 M KCl. The integrase is then concentrated by ammonium sulphate precipitation and finally fractionated by Sephadex G-100 chromatography. During the purification integrase is monitored solely on the basis of its mobility on SDS gel electrophoresis (see legend to Fig. 1 for details). Gel electrophoresis of radiochemically pure integrase is shown in Fig. 1b. The radiochemical purification of integrase represents approximately a 100-fold physical purification. However, the most purified fractions still contain a 100 to 1,000-fold excess of unlabelled proteins.

Depending on its state of purification and on the ionic strength of the buffer, integrase exists in one of three forms, characterised in part by markedly different sedimentation rates. Following sonication, integrase is found in a hetero-disperse collection of large (>100S) particles. These particles contain DNA; treatment with pancreatic DNase disperses the large particles into species of 35S or smaller. After purification to homogeneity, all of the integrase sediments in low ionic strength (<0.1 M KCl) as a fairly homogeneous peak at 35S with a broad tail at low molecular weights. It is unlikely that the 35S form is an integrase-DNA complex since treatment of the 35S form with pancreatic DNase has no effect on its sedimentation properties.

Both the large integrase particles found in crude extracts and the 35S aggregate are disrupted by high ionic strength buffers. In 0.66 M KCl, regardless of its stage of purification, integrase sediments as a homogeneous peak at S_{20,w} approximately 4.0. A sedimentation rate of 4S corresponds to a globular protein of molecular weight 60,000. Since the monomer molecular weight of integrase is 44,000 as determined by SDS gel electrophoresis, the 4S form could either be a monomer or a dimer.

Formation of the 35S aggregate is reversible: high salt (0.3 M KCl) disrupts the aggregate, but the aggregate is reformed on return to low ionic strength. This indicates that 35S aggregate formation is an intrinsic property of integrase which has not been lost due to denaturation.

Since integrase catalyses a site-specific recombination event *in vivo*, radiochemically purified integrase might inter-

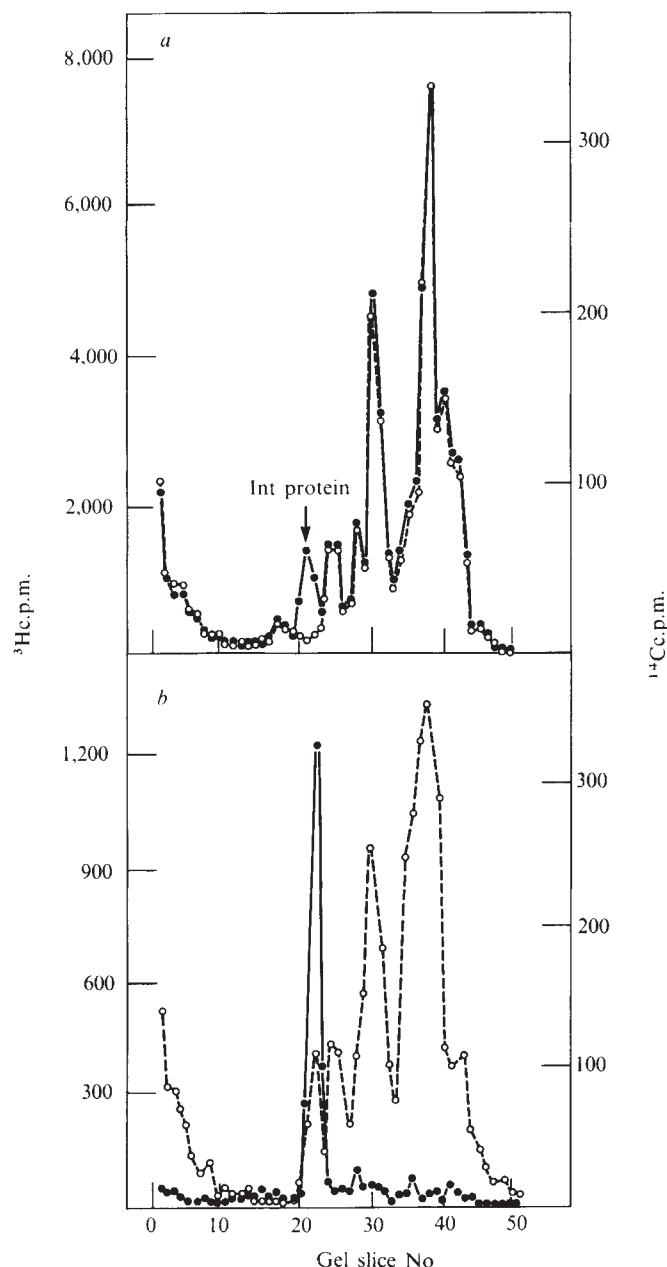


Fig. 1 Electrophoretic identification and radiochemical purification of integrase. *Escherichia coli* strain 159 (*su-gal-str-uvr*⁻) was grown at 37° C to a density of 3×10^8 ml⁻¹ in I medium (0.37% Na₂HPO₄, 0.3% KH₂PO₄, 0.25% NaCl, 0.00075% FeCl₃, 0.4% maltose, 0.001 M MgSO₄). The culture was chilled and then irradiated in 120 ml aliquots in an 18 × 8 inch dish with two 15 watt GE germicidal bulbs at a dose of 8,400 ergs mm⁻². Ten 100 ml aliquots of the irradiated culture were added to ten prewarmed flasks containing 1 mCi ³H-leucine (50 Ci mmol⁻¹) and 2 ml 1.0 M MgSO₄, and infected with λ Eam4b2red β am270c-1857Sam7 at a multiplicity of five particles per cell. Two further 100 ml aliquots of irradiated cells were treated in the same way, except that one was labelled with 25 μ Ci ¹⁴C-leucine (250 mCi mmol⁻¹) instead of ³H-leucine and the other was infected with λ Eam4b2intam29red β am270c1857Sam7 and labelled with 25 μ Ci ¹⁴C-leucine (250 mCi mmol⁻¹). After incubation for 1 h at 37° C, the combined ³H-labelled cultures and the two ¹⁴C-labelled cultures were washed repeatedly with cold I medium and each resuspended in 5 ml TMES buffer (0.01 M Tris, pH 7.5, 0.001 M MgCl₂, 0.0002 M EDTA, 0.1 mg ml⁻¹ dithiothreitol). Each culture was sonicated with cooling for 1.5 min at 60 W and then centrifuged at 10,000 r.p.m. for 10 min to remove debris. The ³H-labelled lysate was further purified by sedimentation into a 2 ml 50% glycerol cushion in TMES buffer at 4° C for 3 h at 40,000 r.p.m. in an IEC SB283 rotor. The glycerol cushion was brought to 0.67 M KCl and 10% glycerol and then spun at 4° C for 12 h at 25,000 r.p.m. in an IEC SB110 rotor onto a 23 ml 40% glycerol cushion containing 0.67 M KCl and TMES buffer. The supernatant fluid above the cushion was collected and brought to 75% saturation with cold, neutralised, saturated ammonium sulphate. After incubation overnight at 4° C, the precipitate was collected by centrifugation at 4° C for 30 min at 18,000 r.p.m., resuspended in 2 ml TMES buffer supplemented with 50 μ g ml⁻¹ bovine serum albumen (BSA) and 10% ethylene glycol, applied to a 1.5 × 30 cm Sephadex G-100 column equilibrated with the resuspension buffer, and then washed through with the same buffer at 4° C at a flow rate of 10 ml h⁻¹. The fractions containing material excluded from the column were pooled and found to contain 60–80% of the integrase applied to the column. The yield from crude extract was 35%. Integrase was monitored during purification solely on the basis of its mobility on sodium dodecyl sulphate (SDS) gel electrophoresis. This was made possible by the construction of special phages containing a combination of amber mutations (*Eam4* and *red β am270*) and a deletion (*b2*) such that they synthesised no other proteins with an electrophoretic mobility near that of integrase. To ensure the correct identification of integrase during the purification, each SDS gel of a ³H-labelled fraction was internally calibrated with unfractionated ¹⁴C-labelled *int*⁺ or *intam* lysate. In preparation for gel electrophoresis equal aliquots of ³H-labelled fractions and unfractionated ¹⁴C-labelled lysate were combined and incubated at 90° C for 5 min with 0.2% SDS and 0.1% dithiothreitol. A 0.05 ml sample of this mixture was subjected to electrophoresis on a 5 × 0.6 cm 10% acrylamide gel. *a*, Un-fractionated ³H-labelled *int*⁺ lysate (●) plus unfractionated ¹⁴C-labelled *int* amber lysate (○); *b*, purified ³H-labelled integrase (pooled G-100 fraction) (●) plus unfractionated ¹⁴C-labelled *int*⁺ lysate (○). Electrophoresis is from left to right.

act specifically with DNA which contains the phage attachment site. To test the possibility of specific *in vitro* binding, radiochemically pure integrase was cosedimented with DNA in glycerol gradients or incubated with DNA and then filtered through nitrocellulose filters.

Integrase in the 4S form does not bind to DNA either specifically or non-specifically, probably due to the relatively high salt concentration (>0.3 M KCl) necessary to maintain the 4S state. On the other hand, when 35S integrase aggregates are mixed with a large molar excess (500–2,500-fold) of 32S λ DNA, the integrase sediments with the DNA at approximately 32S. The same result is obtained with both wild type DNA and with DNA which has the attachment site deleted (*att* ∇). These results suggest that 35S integrase binds non-specifically to λ DNA, and further, that this binding disrupts the 35S aggregate. This interpretation was tested by the following experiment. Integrase aggregates (35S) were cosedimented with a mixture of whole *att*⁺ DNA and sonicated *att* ∇ DNA (and *vice versa*). If the 35S aggregates disperse when binding non-specifically to DNA, then a significant amount of integrase should sediment with

the sonicated *att* ∇ (or sonicated *att*⁺ DNA) at 5S. The result of this experiment is shown in Fig. 2: after sedimentation, the integrase partitioned almost equally between the *att*⁺ and *att* ∇ DNAs. As above these results give no indication of specific binding and, in addition, make it seem unlikely that a relatively weak non-specific binding is obscuring a much stronger specific binding to the attachment region. There was no increase in binding specificity over a wide range of salt concentrations and in various buffer systems containing different cations.

In addition to binding to λ DNA in glycerol sedimentation gradients, radiochemically pure 35S integrase aggregates bind λ DNA to nitrocellulose filters. The filter-binding activity in the preparations was shown to be due specifically to integrase since material from an *int* amber infection, carried through the same stages of purification as the *int*⁺ lysate, did not display any filter-binding activity. Integrase displayed the same amount of filter-binding activity with both *att*⁺ and *att* ∇ DNA.

The experiments described here demonstrate that partially purified integrase binds non-specifically to λ DNA and

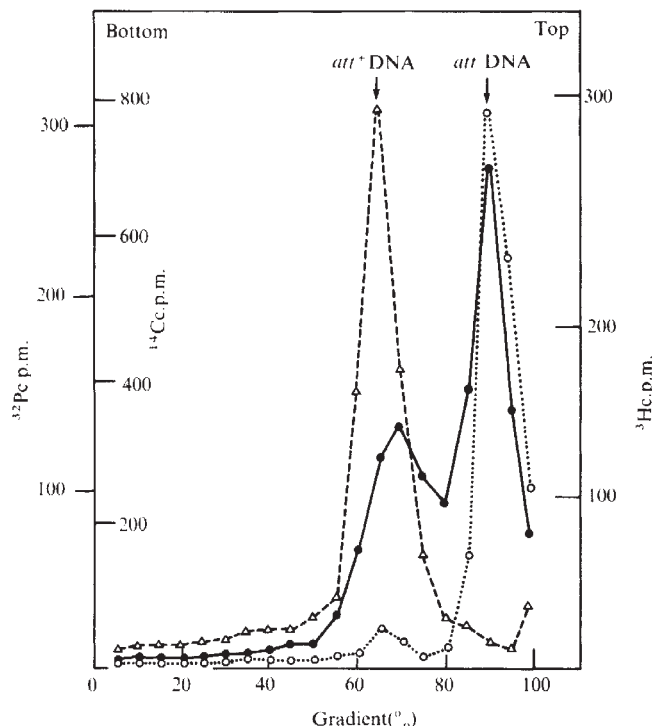


Fig. 2 Cosedimentation of 35S integrase with whole *att*⁺ and sonicated *att*[∇] λ DNA. A 50 μg ml⁻¹ solution of ¹⁴C-labelled *λ*b104*cI*857*Sam*7 DNA (*att*[∇]) was sonicated with cooling for 2 min at 60 W in 0.01 M Tris, pH 7.5, and 0.001 M EDTA. A 25 μl aliquot of radiochemically purified integrase in the 35S form (pooled G-100 fractions from Fig. 1) was incubated at 4° C for 1 h with a mixture of 5 μg whole ³²P-labelled *λ*cI857*Sam*7 DNA and 5 μg sonicated ¹⁴C-labelled *λ*b104*cI*857*Sam*7 DNA. The incubation mixture contained 0.01 M Tris, 0.001 M MgCl₂, 50 μg ml⁻¹ BSA, and 10% ethylene glycol. The DNA-integrase mixture was layered on 3.6 ml 10–30% linear glycerol gradient containing the same ingredients as the incubation buffer, and then spun at 4° C for 2 h at 60,000 r.p.m. in an IEC SB405 rotor. The gradient was dripped directly into scintillation vials and counted. When the experiment was repeated with sonicated *att*⁺ DNA and whole *att*[∇] DNA, essentially the same result was obtained, that is, integrase counts partitioned approximately equally between the whole and sonicated DNAs. When integrase was cosedimented only with sonicated *att*⁺ or *att*[∇] DNA all of the integrase sedimented with the sonicated DNAs. ³H-labelled integrase (●); ³²P-labelled *λ*cI857*Sam*7(*att*⁺) DNA (Δ); ¹⁴C-labelled sonicated *λ*b104*cI*857*Sam*7 (*att*[∇]) DNA (○).

reversibly forms a fairly homogeneous aggregate which sediments at approximately 35S. With further purification of integrase it may be possible to demonstrate the existence of an integrase-mediated reaction specific for attachment region DNA and to elucidate the role of the 35S aggregate in this process. One can speculate, for example, that the 35S aggregate functions as a structural cage to hold pairs of attachment sites together.

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Changes in Rates of Increase in Size and of Exoskeletal Production during Old Age in the Isopod *Ligia oceanica* (L.)

THE male isopod *Ligia oceanica* (L.) goes on moulting and growing even when its spermatogenesis has come to an end¹⁻⁴. The coexistence of growth and senescence seems to me an unexpected finding in a higher metazoan. It is possible to split up the postpuberal growth period of *Ligia* into the sexually mature stage and the senescent stage, both of which are states of morpho-physiological equilibrium, and an intermediate transitional phase. The term 'stage' is here used as equivalent to the French term *étape* and is composed of several 'stadia' separated by a moult. In the sexually mature stage the gonads are fully developed, in the senescent stage they are degenerated. The transitional phase is characterized by various degrees of testicular degeneration.

Sexual deterioration is not, of course, the only criterion of senescence, and I have shown⁵ that the respiratory rate falls off strongly during the senescent stage. In another study⁶, weight and calcium content of the exuvium were found to vary more during the senescent stage than during the sexually mature stage. I thought that loss of vitality would thus lead to greater metabolic irregularity of exoskeletal formation and of exuviation and calcification, and wondered whether the senescent animal is different from the younger adult in other quantitative exoskeletal characters than merely greater exuvial variation. I have therefore undertaken a comparative study on the growth rate during the two stages and the transitional phase.

Isopods were collected during the spring tides on the coast of Normandy (Luc-sur-Mer, Calvados, France, in May 1966 and July 1967). Some 200 premoulting adult males of various sizes were then reared in the laboratory at a temperature of 17° C, for two successive moults. Most survived

Table 1 Rate of Increase of Head Breadth

Stage or phase	Number of individuals	<i>R</i>	<i>σ</i>
Sexually mature	27	1.0702	0.00652
Transitional	30	1.0471	0.00691
Senescent	27	1.0155	0.00449

The rate *R* is defined by $R = H_1^{-1}H_2$, where *H*₁ and *H*₂ are the head breadth at two consecutive stadia. $\bar{R}$, geometrical mean of *R*; *σ*, standard deviation of log *R*.

a first moult but only eighty-four survived the second. The head breadth and the dry weight of the exuvium of each individual were measured.

The growth rate from one stadium to the next generally varies with the environment, and in crustacea seems to be lower in the laboratory than in the wild. In the premoult state where the animals were caught, the conditions that determine the characteristics of the moult were, however, already laid down. A comparison of the exuviae of first and second moult, which show sizes of animals at the two successive stadia before and after capture, may therefore give a fair picture of growth rate in the wild.

The treated animals were divided into three groups corresponding to the two stages and the transitional phase. The geometrical mean of the growth rate was calculated for each group (Table 1). The growth rate was markedly lower in the senescent stage than in the sexually mature stage; the difference (reckoned by a *t*-test carried out on the logarithm of the growth state) is highly significant ($P < 0.001$).

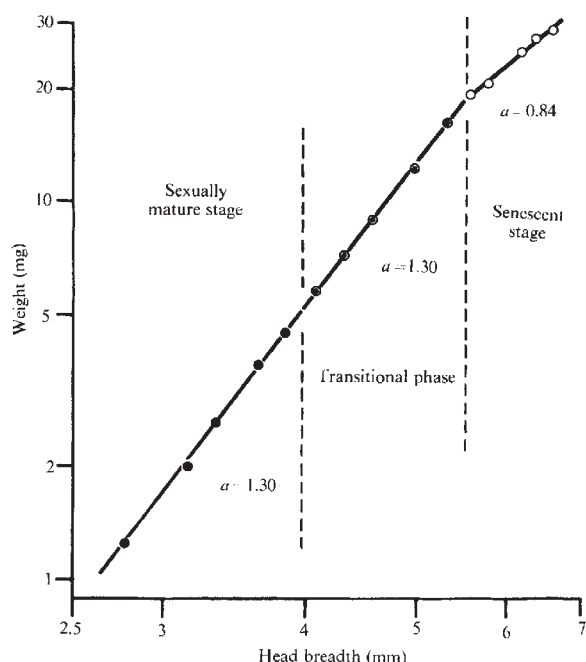


Fig. 1 Relative increase of weight (W) to head breadth (H) of the exuvium. Each point stands for the geometrical mean of ten individuals. a , Regression coefficient of $\log W$ on $3 \log H$.

I then studied the relation of weight (W) to head breadth (H) of the exuvium in 150 individuals, to estimate the rate of exoskeletal production at each stadium. This should give an indication of anabolic intensity and thus of growth capacity in mass. The individuals belonging to each stage or phase were grouped by tens according to their H value. The geometrical means of H and W for each such class were plotted on a double logarithmic grid (Fig. 1). The figure displays a similar growth curve for the sexually mature stage and for the transitional phase, but the curve for the senescent stage is different, with a lower regression coefficient a of $\log W$ on $3 \log H$. Reeve's stochastic test⁷ shows this difference of a to be highly significant ($P < 0.001$). The growth rate in mass may be deduced, from this lower value of a , to fall off with old age. Thus, although an aged isopod can moult and grow, notwithstanding testicular degeneration, its senescence is fairly marked by a fall in the rates of increase of size and mass.

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Lantern Fish compare Downwelling Light and Bioluminescence

THE biological utility of the light organs of oceanic fishes has been the subject of much speculation^{1,2}. According to one hypothesis the ventral photophores of some mesopelagic fishes in the upper regions of the sea may produce bioluminescence that matches the colour and intensity of environmental light passing downwards on either side of the fish. Such a system of camouflage could obscure the ventral outline of the fish when viewed from below^{3,4}.

This hypothesis is supported by several lines of evidence. First, light in the sea is highly directional, the ratio of upward to downward directed light being small (1:200 to 1:100) in clear oceanic waters. Consequently, the reflecting dorsal and lateral surfaces of a fish enable it to match the background when viewed from above and from the side, but the ventral silhouette seen from below would appear dark against the brighter background⁵. Second, the blue wavelengths of the spectrum penetrate most deeply into the ocean, and therefore daytime illumination at depths of 500 to 1,000 m is blue and dim. Bioluminescence of some midwater fishes is blue and falls within the range of intensities measured during the day at these depths and has a very similar angular distribution⁶⁻⁹.

One species of midwater fish has proved invaluable in the study of this hypothesis and its implications. *Tarleton-beania crenularis* is a common lantern fish (Myctophidae, Iniomi) living in the North Pacific Ocean in deep water beyond the continental shelf. Unlike many other mesopelagic fish, this species migrates to the surface at night from daytime depths of 500 to 1,000 m and is often associated with sonic scattering layers¹⁰⁻¹². Lantern fishes are bioluminescent. Light organs or photophores occur in rows along the ventral surface of the head and body and groups of isolated organs may be found around the eyes. Each dermal photophore consists of a mass of innervated photogenic tissue backed by a pigmented mantle and reflector. Some light organs are covered by a superficial lens, scale and reflector system which concentrates and collimates the light from the photogenic tissue and modifies the colour and intensity of the emitted light¹³⁻¹⁵.

If the ventral surface of a lantern fish is to remain invisible in the face of changing intensities of incident light, the fish should be able to monitor both the environmental illumination and the activity of its photophores. *Tarleton-beania crenularis* may see downwelling light and bioluminescence from a supraorbital photophore, and adjust the output of its ventral photophores to match environmental illumination.

Lantern fishes swimming near the surface on calm, dark nights are attracted to an underwater light and can be collected with dipnets and maintained in fairly good condition in darkened aerated aquaria for several hours. Fishes kept in total darkness rarely luminesced, but when they were illuminated from above with blue light the ventral photophores of some specimens emitted a bluish light

lasting for about 15 min which approximated the colour and intensity of the incident illumination and which was extinguished when the incident illumination was turned off. The bioluminescence was only visible after about 30 min of dark adaptation on the part of the observer when the intensity of the incident light was very dim. It was not possible to determine whether the supraorbital photophore was luminescing simultaneously with the ventral photophores as the bioluminescence very closely resembled the colour and intensity of the incident light, and the relationships of the orbital photophores to the eyes of *Tarletonbeania* were studied for evidence of a feedback loop between the bioluminescent and visual systems.

The eyes of lantern fishes are large and well adapted for vision in dim light. The nearly spherical lenses of a 41 mm ♀ *Tarletonbeania* were 1.3 mm in diameter and protruded well through the pupils; the centres of the lenses were nearly in line with the iridial margins and coincident with the centres of the pupils (Fig. 1). The protruding lenses of fishes (refractive index 1.67) compensate for the absence of corneal refraction, since corneal tissues have the same refractive index (1.33) as water¹⁴.

The eye of *Tarletonbeania* is particularly distinguished by the presence of a crescent shaped gap or aphakic aperture between the central margin of the iris and the ventral border of the lens. This aperture has not been described in lantern fishes before but is found in other bathypelagic species¹⁵. The aphakic aperture of *Tarletonbeania crenularis* is so placed that light rays from the supraorbital

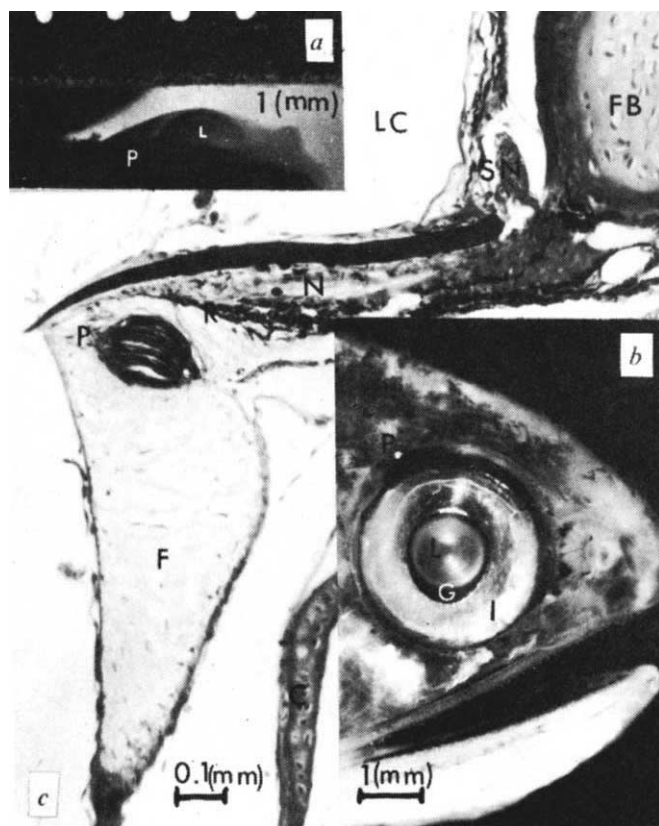


Fig. 1 Structure of the eye and supraorbital photophore of the lantern fish, *Tarletonbeania crenularis*. *a*, Dorsal view of the left eye of a 41 mm ♀ specimen, showing the protruding lens, L, subjacent to the cornea, and P, the position of the supraorbital photophore. *b*, 10 µm paraffin section stained with Mallory triple connective tissue stain; F, transparent fold of tissue in which photophore, P, is located; R, reflector; N, branch of supraorbital nerve; SN, innervating the photophore; FB, frontal bone; LC, supraorbital lateral line canal; C, cornea. *c*, A lateral view of eye of *Tarletonbeania* showing the supraorbital photophore; P, (the fold of tissue over photophore has been removed); L, lens; G, aphakic aperture between lens and the iridial margin; I, iris.

photophores and from the dorsal visual fields can reach the retina (Fig. 2).

The supraorbital light organ (depicted in figures of Bolin¹⁸, but not described) is situated in a flap of tissue at the posterodorsal margin of the orbit. This organ is innervated by a branch of the supraorbital nerve and is enveloped posteriorly and medially by a reflector and pigmented epithelium (Fig. 1b)¹⁶.

Light from the supraorbital photophore falls on the posterior-superior surface of the lens and the anterior-inferior quadrant of the retina. The approximate paths of light rays from the supraorbital photophore and from the dorsal visual fields through the lens and onto the retina were constructed ignoring corneal refraction. The distances from the supraorbital photophore to the surface of lens, the diameter of the lens, and the size of the aphakic aperture were measured from photographs of dissected preparations and histological sections. An almost constant feature of

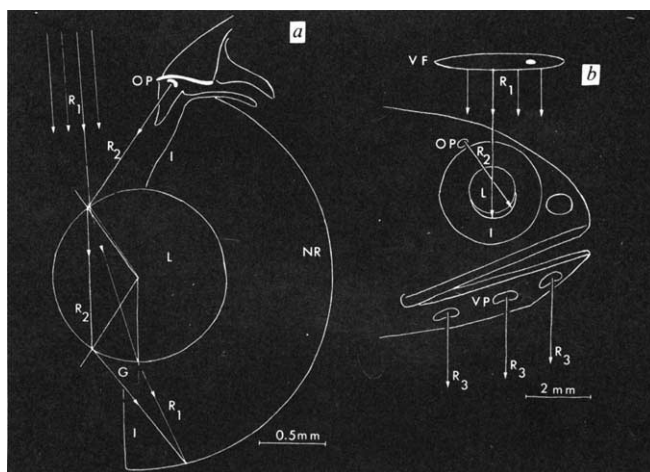


Fig. 2 Optical paths taken by light from the dorsal visual field and from the supraorbital photophore of *Tarletonbeania crenularis* through the dioptric media of the eye. *a*, Diffuse downwelling light from the dorsal visual field, R_1 , and a ray of light, R_2 , from the supraorbital photophore, are bent by the lens, L, and fall on the neural retina, NR. Some light passes through an aphakic aperture, G, between the iris, I, and the lens. *b*, A lateral view of light rays R_1 from the dorsal visual field, VF, and R_2 from the supraorbital photophore, entering the eye through the aperture between the lens, L, and the iris, I. The fish may see a spot in the visual field cast by light from the supraorbital photophore, if the intensities of R_1 and R_2 are unequal. If the fish adjusts the intensity of R_2 to equal R_1 and controls the output, R_3 , of the ventral photophores, VP, accordingly, then the ventral body outline will be camouflaged maximally.

fish eyes is the ratio of the distance from the centre of the lens to the retina divided by the radius of the lens. This ratio (Matthiessen's ratio) is about 2.55 and it was used to obtain the distance from the centre of the lens to the retina to compensate for errors in measured distances created by artefacts of fixation. Snell's law and a value of 1.67 for the refractive index of the lens were used to determine the angles of refraction at the surfaces of the lens¹⁷⁻¹⁹. The constructed path of light rays from the supraorbital photophore was confirmed by splitting the head of a fresh fish, dissecting the orbit away from the back of the retina, and directing a beam of light from a fibre optic system from the anterior retinal quadrant backwards through the aphakic aperture and lens and onto the supraorbital photophore.

These findings suggest that *Tarletonbeania crenularis* can see a diffuse spot of light cast by the supraorbital photophores in the dorsal visual fields whenever the intensity of the light from the photophore is greater than that of the dorsal visual field. It might be supposed that the fish can perform an incremental threshold discrimination of intensity

and adjust the output of the supraorbital photophore until the spot is obliterated. If the fish controls the intensity of light from the ventral photophores accordingly it will not be visible from below against changes in downwelling illumination.

For the photophore system to be maximally effective in camouflaging the ventral body silhouette, a luminescing fish should be horizontally orientated. Observations from bathyscaphes^{20,21} show that some non-luminescing myctophids were orientated in positions other than the horizontal.

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Rate of Uptake of DDT from Soil by Earthworms

SINCE 1958, when the occurrence of DDT and its metabolites in the tissues of earthworms was first reported, there have been more than twenty other reports of DDT residues being found in these animals^{1,2}. The amounts found have differed greatly, ranging from minute traces to concentrations of nearly 700 p.p.m., although concentrations of the order of 1 to 10 p.p.m. have been more commonly reported. Sometimes the concentrations of DDT residues found in the earthworms have been less than in the soil where they live, although the tissues of the worms have usually contained greater concentrations than the surrounding soil. When all the published data were considered, it was seen that there is a linear correlation between the concentration of DDT (and other organochlorine insecticides) in earthworms and that in the corresponding soil¹. Not all species concentrate insecticides in the same way or to the same degree³; *Lumbricus terrestris* and other species with permanent burrows take up DDT from the soil surface more readily than from residues that have been thoroughly mixed into the upper strata of soil. Conversely, some of the species without permanent burrows, that tunnel haphazardly through the upper 150 mm of soil, take up more residues from insecticides evenly distributed through the upper soil layers to plough depth.

One limitation of the published data is that, in most in-

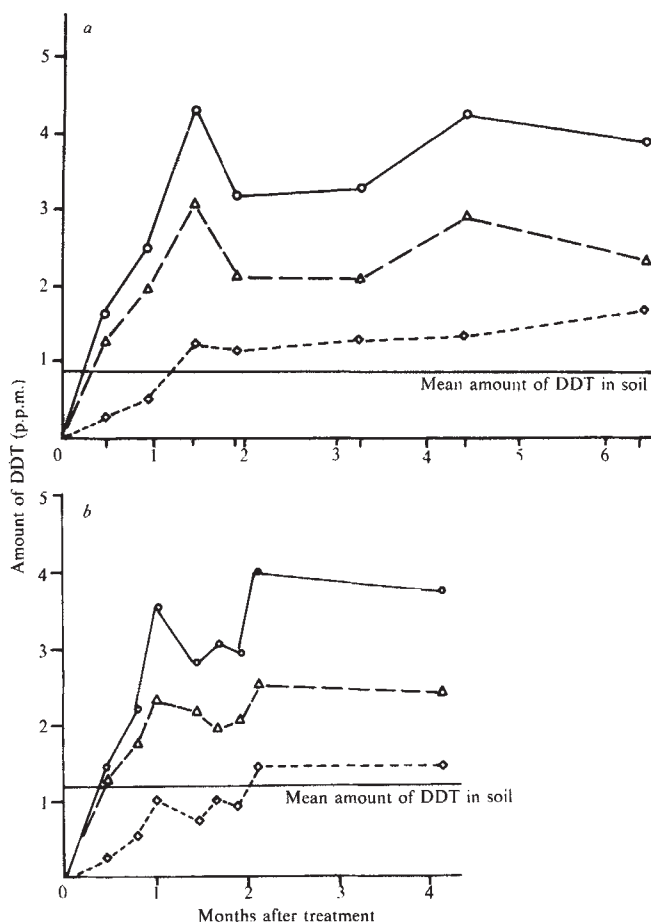


Fig. 1 Results of two series of uptake experiments. $\circ$, DDT and DDE; $\triangle$, DDT; $\diamond$, DDE.

vestigations, residues in samples of soil and earthworms were taken at some arbitrary point in time, without defining the period during which the worms were exposed to soil insecticide residues; so such random samples may represent any stage in what is possibly a long process of absorption and concentration. The rate of uptake or excretion of DDT by earthworms has not been studied.

We therefore investigated the process in a series of laboratory experiments. Uptake of DDT into earthworms was followed in twelve culture boxes (450 mm high $\times$ 180 mm wide $\times$ 90 mm deep) with wooden sides and perspex backs and fronts that could be easily removed, containing DDT-treated soil. Each box contained ten healthy *Lumbricus terrestris*. This soil, which was free from insecticides before treatment, was passed through a 6 mm aperture sieve to remove stones and debris and was then spread out on a large plastic sheet, sprayed with DDT through an atomiser and thoroughly mixed before being placed in the culture boxes. The dose of DDT was calculated to give approximately 1 p.p.m. ($\mu\text{g g}^{-1}$) in the soil. Four worms were taken from culture and put separately into Petri dishes containing moist filter paper for 24 h, to allow their gut contents to be voided, then stored at 15° C for several days before being analysed for residues. Small soil samples were taken from each box at the same time, and bulked before extracting the DDT residues.

The excretion of DDT from worms that had lived in soil containing DDT for their entire life cycle was studied by taking sixty such worms from contaminated soil in the field and placing them in culture boxes containing uncontaminated soil. At intervals, batches of four worms were taken at random from the boxes and residues analysed in the same way as in the uptake experiments.

Residues were extracted by grinding frozen worms with four times their weight of anhydrous sodium sulphate, and tumbling

for 2 h with a mixture of 100 ml of acetone and 100 ml hexane in polythene-capped jars. The extracts were left to stand until the sediment settled, filtered through a Gooch funnel, transferred to a separating funnel and shaken with 100 ml 2% sodium sulphate solution. The aqueous phase, which settled at the bottom of the funnel, was run off and the remaining hexane/acetone extract stored at 0° C with a little anhydrous sodium sulphate until analysed by gas-liquid chromatography (GLC).

The soil from which the earthworms were taken was analysed for DDT by grinding 50 g of the soil, taken in many small subsamples from the four boxes, with 50 g sodium sulphate, and tumbling in an acetone/hexane mixture for 4 h. The subsequent procedure was the same for the worm extracts.

Small aliquots of the soil or worm extracts were concentrated in a Kuderna-Danish evaporator, and passed through a column of Celite impregnated with a sulphuric acid-oleum mixture to remove most of the interfering substances, but not DDT or DDE which is the principal degradation product. The amounts of DDT and DDE in the final extract were determined by GLC using a stainless steel column (1 m long, 3 mm diameter) packed with 5% SE30 on Chromosorb W and operated at 185° C. An electron-capture detector was used with nitrogen as a carrier gas.

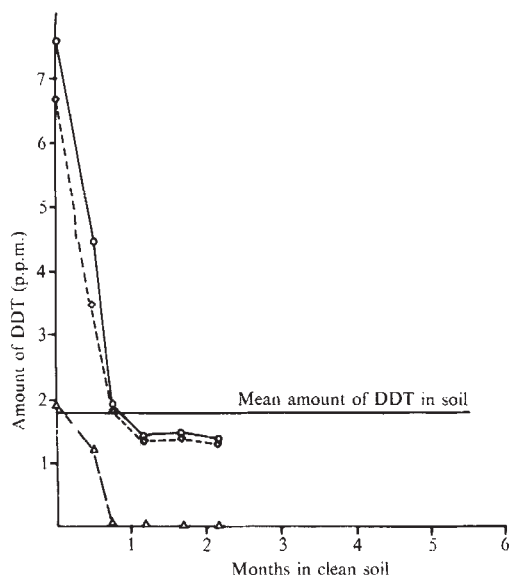


Fig. 2 Results of the excretion experiment. Symbols as in Fig. 1.

The results of the uptake experiments are summarised in Fig. 1a and b and those of the excretion experiment in Fig. 2. Because the earthworms were progressively removed for analysis or died, it was impossible to continue the experiments for more than 6 months. This period was, however, sufficient to show that worms accumulated the maximum amount of DDT residues from soil after about 1 month; thereafter, although there were small fluctuations, the amounts did not change greatly. There seemed to be three phases—an initial fairly rapid uptake followed by some loss, then a further uptake before equilibrium was reached. Similar double maxima have been reported for the uptake of DDT by other animals such as snails⁴ and aquatic organisms⁵. The explanation has generally been that the initial rapid uptake is due to ingestion, whereas the slower phase represents redistribution or residues within the organism, but this needs confirmation. There was evidence that in the box experiment conversion of DDT to DDE within the worms continued and this was confirmed with worms taken from the field; in the latter there was much more DDE than DDT. Moreover, worms placed in untreated soil excreted DDT rapidly, but some of the DDE persisted in their tissues for several months. Some workers have reported larger amounts

of DDT than DDE in worms^{3,6-8}, but others found either the reverse⁹ or that there could sometimes be more DDE and sometimes less¹⁰.

There seems little doubt from our experiments that DDE persists longer in worm tissues than DDT. The presence of DDE in worms indicates that they contribute significantly to the initial degradation of DDT, because the amount of DDT converted to DDE in soil is small, possibly only of the order of 10% over several months or even years. During the period of our experiments there was virtually no conversion of DDT to DDE in soil (only traces of DDE being found in the extracts), whereas nearly half the DDT taken up by worms was converted to DDE in 4 to 6 months; and in worms that had been exposed to DDT for a long time, nearly all the residues were DDE.

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Effects of Interrupted Light on Plant Disease

RESULTS of experiments with scab disease on MM109 apple rootstock shoots showed a marked lack of reproducibility, even when done in a glasshouse with some control of light and humidity as well as temperature (the mean visible light intensity was controlled to $1,300 \pm 200$ cal per cm^2 per month¹, ambient temperatures to $20 \pm 2^\circ$ C during the day and $15 \pm 2^\circ$ C at night with corresponding relative humidities of $70\% \pm 5\%$ minimum and $90\% \pm 5\%$ maximum). The disease system seemed to be very susceptible to residual fluctuations in the environment. Although this situation could be partly attributed to mechanical failures, examination of results and sunlight radiation records collected over a period of years indicated that erratic and short reductions in sunlight intensity, due to small cumulus clouds passing during the end of the first week after infection, could account for a considerable part of the irregularity. This study, though stimulated by previous observations on apple scab disease, was done with *Venturia inaequalis* and *Podosphaera leucotricha* on MM109 apple rootstock shoots, and strawberry latent ring-spot virus on cucumber plants, in order to investigate the influence of interrupted light on plant disease generally.

Groups of inoculated plants were separated by opaque partitions under a timber-framed hood covered with black polythene to exclude approximately 90% of the daylight, and were illuminated by wide spectrum Kolorarc lamps (Phillips Ltd). Plants received continuous unmodulated light during the day, or were shaded at intervals for 2-min periods by the passage of automatically controlled reciprocating metal shutters, which excluded approximately 90% of the incident light when closed. All plants had at least 12 h of uninter-

ted light on the day prior to inoculation. The cucumber plants were subjected to a 16 h day, and when shaded received two, four, or six periods per h every day for 7 d after inoculation because of the rapid onset of the virus disease (see Fig. 1).

Before inoculation of the apple rootstock shoots, the first fully unrolled and partially expanded leaf below each shoot tip was labelled on the petiole and designated Lo, the two leaves above being +1 and +2 and the two below -1 and -2. After inoculation with either *V. inaequalis* or *P. leucotricha*, the following light treatments were applied during 12 h days: (a) unmodulated light for the first 6 d; (b) unmodulated light for the first 4 d followed by 2-min shaded periods twice per h during the fifth and sixth days; (c) as (b) but with four 2-min shaded periods per h; (d) as (b) but with six 2-min shaded periods per h. The plants were subjected to normal glasshouse conditions for the rest of the test period. For both the diseases the numbers of lesions appearing on the five recorded leaves of each shoot were counted during the tenth to the twenty-first days after infection. Light intensities were adjusted in all experiments to compensate for the effects of the shaded periods. Leaf temperature changes in these conditions were comparable

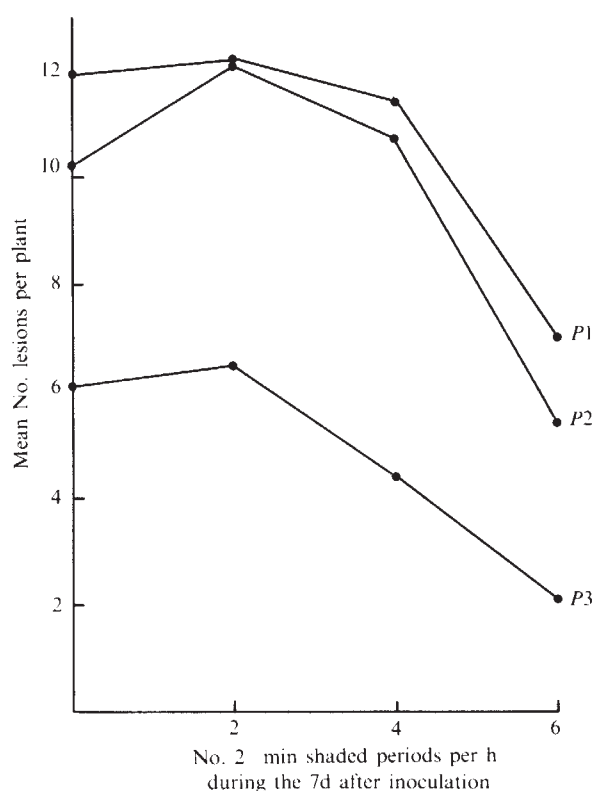


Fig. 1 Inhibition of strawberry latent ringspot virus disease on cucumber plants by the application of six 2-min shaded periods per hour. (Experiment repeated three times for which P_1 , P_2 and P_3 are <0.001 , respectively.) Cucumber seedlings (cultivar, Ohio), potted singly into 3-inch plastic pots, were inoculated on the cotyledons 7 d after sowing. A strain of strawberry latent ringspot virus (X/XX/XS/SS/Ne Nepo virus group), isolated from blackberry, was maintained in cucumber source plants. Young leaves from plants with symptoms were collected 40 d after inoculation and ground with a pestle and mortar (1 g leaf to 5 ml of 0.03 M phosphate buffer, pH 8). The inoculum was rubbed on to cotyledon leaves using the forefinger, plants being taken singly from the different treatment groups in turn, between 1000 h and noon. The cucumber leaves were then washed with distilled water and placed immediately under their respective treatments. Seven days after inoculation a count was made of the number of chlorotic primary lesions, which were best seen by transmitted light. There was no diminution of disease under unmodulated light when the total light received was reduced by the same amount either by day length or light intensity. These effects were readily reproduced.

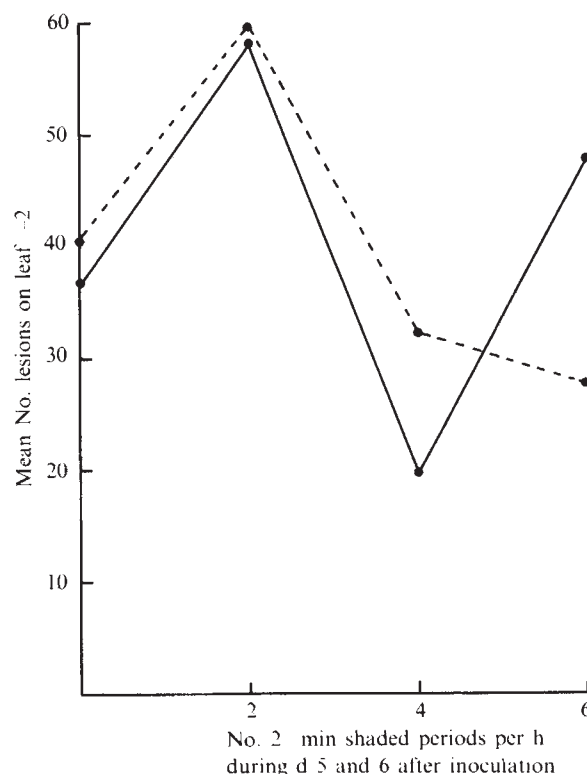


Fig. 2 Effects of interrupted light on the incidence of mildew on apple leaves approaching maturity at the time of infection. ●—●, Control; ●---●, kinetin. Plants were placed in an inoculation tower for mildew infection² 24 h before inoculation and removed 18 h afterwards. Inoculum was obtained from four to six heavily infected apple rootstock shoots, to give well-spaced lesions². Kinetin solution (60 mg l⁻¹) was applied to each shoot as a spray at the rate of 3 ml per ten plants, 2 and 3 d after inoculation. The disease was greatest when the shaded periods were applied twice per hour, and least when applied four times per hour ($P<0.001$). Intermediate effects occurred on plants receiving continuous light or six shaded periods per hour during the day. This result was reproducible on either primary or secondary shoots. Kinetin altered the response of the disease to the effects of six shaded periods per hour ($P<0.05$).

with those occurring in naturally modulated sunlight. The mean visible light received by plants while being treated was 30 ± 5 cal per cm² per day.

The effects of light interruptions on disease reported here, we think for the first time, were impressive yet quite different on each of the systems tested. Experiments with the two obligate parasites gave results which were readily reproducible, in contrast to those given by *Venturia inaequalis*. This facultative parasite, although very susceptible to the way in which light is received, seems to be influenced by other environmental factors which make reproducibility difficult to achieve. The effects of temperature during infection, and of preinoculation conditioning of the plants, are under investigation. The scab and mildew experiments showed the effects of light interruptions to be most marked on leaves approaching maturity at the time of inoculation, though they were visible on all the recorded leaves.

Previous work showed that kinetin can alter both the amount and vertical distribution of the scab disease on the apple shoot, strongly suggesting that the disease is affected by the hormone system of the host^{4,5}. The effect of kinetin on apple plants with mildew, when subjected to six light interruptions per hour during the fifth and sixth days after infection, supports the previous suggestion and also indicates that the response to interrupted light is hormone-dependent.

Many plant diseases respond to changes in photoperiod, which in the long term will alter the amounts of available photosynthate. Thus the application of long dark periods both before and after infection affected the growth of bacterial leaf blight on bean, cucumber, cotton and soybean⁶.

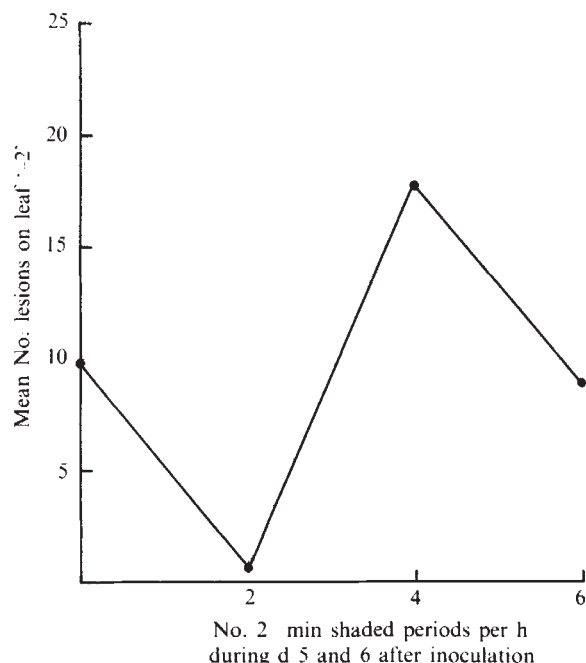


Fig. 3 Effects of interrupted light on the incidence of scab on apple leaves approaching maturity at the time of infection. Cultures of *Venturia inaequalis* (Clone E1)³ grown on filter paper cylinders standing in 10% (w/v) malt extract solution were used as the source of inoculum in the apple scab experiments. Each shoot was individually sprayed, using compressed air, at the rate of 3 ml per ten plants, the inoculum density being approximately 50,000 conidia per ml⁴. The most striking effects occurred after the application of two, or four, 2 min-shaded periods per hour ($P < 0.001$). Seventy-five per cent of the leaves were apparently immune after receiving two shaded periods per hour and the maximum number of lesions seen on a leaf after treatment was five. By contrast, shading applied four times per hour resulted in only 25% of the leaves showing no disease and a maximum of forty-seven lesions on a leaf.

Young leaf disks inoculated with tobacco mosaic virus produced more virus under the influence of increased light whereas older disks did not respond⁷. The development of *Verticillium* wilt in sunflower was reduced by shortening the day length, but was restored to normal when the light deficit was eliminated by a period of illumination applied during the night⁸. Light also affects the hormone system of plants as indicated by the control of apical dominance in *Phaseolus vulgaris*⁹ and by flower initiation in several plant species when light is applied as an intermittent supplement¹⁰. Since in our experiments the total amounts of light received by the plants were approximately equalised between treatments, thereby reducing variation in amounts of photosynthate, the results indicate that the disease responses to 2-min shaded periods were a function of the hormone systems.

We conclude that the reproducibility of quantitative experiments with many plant diseases will depend upon controlled modulation of incident light, in addition to the accepted standards of light intensity, temperature and humidity. Controlled modulation of incident light may also provide a subtle way of regulating disease development in the study of host-pathogen relations after infection.

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Metabolic Adaptation in the Manic-Depressive

WHEN the manic-depressive is psychologically disturbed, biogenic amine metabolism¹, adrenal cortical activity², the disposition of sodium³ and the concentration of cyclic AMP in the urine⁴ may be modified. During normothymic intervals none of these measures of endocrine function fall outside of normal limits¹⁻⁴. Occasional and temporary diabetic-like responses to a glucose tolerance test appear, however, not only during episodes of depression and mania, but also during asymptomatic periods, despite an adequate carbohydrate intake and with no discernible impact on affect⁵. If, as we have suggested⁶, manic-depressives have a defect in the regulation of hormonal balance, an environmentally induced perturbation might make the difficulty apparent even when they are normothymic and their response to a glucose load is normal.

In a healthy man, fasting provokes changes in hormone balance⁷ and, probably, changes in the activities of a number of adaptive enzymes⁸. The response to a glucose load is diabetic-like in contrast to the normal response before the fast. A group of manic-depressives was therefore subjected to this simple and reasonably innocuous test of their ability to reorganise their hormonal balance when faced with an environmental demand for such a change.

Sixteen males volunteered for a 3 d fast. All had glucose tolerances that were non-diabetic as defined by the US Public Health Service⁹. Six were staff members, five were manic-depressives who were normothymic at the time of the experiment, and five were schizo-affectives. The latter groups were being treated with lithium carbonate and were selected from a population whose metabolic and psychological states had been followed for at least 3 yr. Diagnoses were based on the presence of the following symptoms: manic states—hyperactivity, flight of ideas, pressure of speech, elated or irritable mood; depressed states—motor retardation, poverty of thought, depressed mood; schizo-affectives—disintegrated thought and affect and concomitant changes in mood. All of the manic-depressives had a history of at least three manic episodes and two depressed episodes. All of the schizo-

Table 1 Experimental Protocol

Days 1-3	Diet containing at least 250 g carbohydrate. No food after 2200 h on day 3.
Day 4	Pre-fast GTT*—Oral test starting at 0800 h. (Blood samples were collected in the fasting state, then 0.5, 1, 2 and 3 h after drinking a solution containing 75 g of glucose.) No food for remainder of day.
Days 5-6	Fast.
Day 7	Fast GTT*—Repeat oral test starting at 0800 h, then diet containing at least 250 g carbohydrate.
Days 8-9	Diet containing at least 250 g carbohydrate. No food after 2200 h on day 9.
Day 10	Refeed GTT*—Repeat oral test starting at 0800 h.

* GTT—Glucose tolerance test.

Table 2 Age, Weight and Lithium Consumption of Participants

Subject	Age	Li ₂ CO ₃ dosage (mg)	Height (inches)	Weight * (pounds)		
				Pre-fast	Fast	Refeed
Controls						
RH	35	—	73	170	165	167
RT	22	—	71	149	142	145
AB	55	—	68	163	157	160
LV	50	—	75	202	193	197
BM	31	—	76	152	145	148
EF	46	—	67	158	153	156
Manic-depressives						
WG	22	600 bid †	67	143	135	139
AS	30	600 qid †	70	202	194	199
BH	51	300 qid	75	284	272	279
LD †	47	—	71	197	187	191
LD †	47	600 bid	71	202	191	196
PM	28	600 bid	69	155	149	153
Schizo-affectives						
AM	23	300 qid	66	179	171	175
CW	45	300 qid	69	162	156	160
FD	34	600 bid	74	218	206	213
RB	23	600 bid	72	222	212	219
SE	23	300 qid	71	180	174	178

* Participants were weighed just before the glucose tolerance tests on days 4, 7 and 10.

† Bid, twice daily; qid, four times daily.

‡ Patient took test twice: once after he had discontinued lithium carbonate treatment for approximately 3 months; once after he had resumed lithium carbonate treatment for 1 month.

affectives had a history of at least five episodes of mania, hypomania or depression.

The experimental procedure is outlined in Table 1. Participants who had been taking lithium carbonate before the experiment continued their medication in the same amounts and on the same schedule during the fast and refeeding periods (Table 2). This design was selected since several patients were reluctant to discontinue lithium therapy. One patient (LD) was tested with and without lithium. No significant affective changes took place in any of the participants during the experiment.

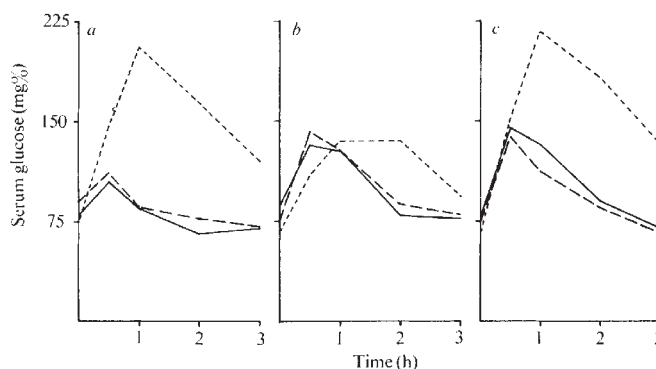
Serum glucose was determined by the hexokinase: glucose-6-phosphate dehydrogenase reaction¹⁰. Serum lithium was measured by atomic absorption spectrophotometry and sodium and potassium with a flame photometer. Serum lithium levels increased during the fast but the decrease in

Table 3 Levels of Monovalent Cations in Serum (mEq l⁻¹)*

Subject	Sodium			Potassium			Lithium		
	Pre-fast	Fast	Refeed	Pre-fast	Fast	Refeed	Pre-fast	Fast	Refeed
Controls									
RH	143	139	143	4.7	4.2	4.4	—	—	—
RT	146	140	144	4.5	4.1	4.3	—	—	—
AB	144	141	145	4.3	4.1	4.2	—	—	—
LV	147	141	146	4.7	4.4	4.6	—	—	—
BM	144	143	147	4.2	4.0	4.3	—	—	—
EF	142	140	143	4.3	4.1	4.3	—	—	—
Manic-depressives									
WG	145	140	145	4.3	4.2	4.4	0.65	0.98	0.58
AS	144	139	143	4.3	3.9	4.0	0.87	1.35	0.78
BH	142	140	143	4.7	4.4	4.6	1.11	1.40	0.83
LD†	145	142	144	4.2	4.0	4.3	—	—	—
LD†	146	142	145	4.3	4.1	4.3	0.73	1.16	0.91
PM	146	140	145	4.5	4.2	4.4	0.98	1.24	0.97
Schizo-affectives									
AM	146	141	145	4.7	4.2	4.4	0.99	1.45	0.91
CW	148	145	149	4.9	4.3	4.8	0.71	1.00	0.61
FD	145	143	146	4.0	3.8	4.1	0.60	1.15	0.64
RB	144	143	147	4.4	4.1	4.3	0.70	1.08	0.68
SE	146	140	144	4.3	4.1	4.3	0.77	1.01	0.73

* Ions measured in a portion of the blood samples taken for the zero time glucose tolerance tests.

† See footnote‡, Table 2.

**Fig. 1** Effects of fasting on the response to a glucose load. —, Pre-fast; ---, fast; ···, refeed. a, Control; b, manic-depressive; c, schizo-affective.

serum sodium and potassium was not significantly different from decreases in controls (Table 3). Urine samples were tested for 'ketone bodies' with a commercial preparation that contains sodium nitroprusside and detects acetoacetic acid at a level of 5 mg per 100 ml. A positive reaction appeared on day 6 in all cases and became intense by day 8. No reaction was detected on days 4 or 10.

The results of the glucose tolerance tests are shown in Fig. 1 and Table 4 and, in the case of LD, in Fig. 2. The fast produced notable differences between manic-depressives and the other groups. First, the response to the glucose load, as measured by the sum of the five sampling periods, was lowest in manic-depressives and only slightly higher than their pre-fast levels (36 mg%) while this sum was increased by 306 mg% in the non-medicated group and by 240 mg% in the medicated controls. Second, the half-hour serum glucose levels of the manic-depressives were lower than their comparable pre-fast or refeed levels by almost 20%. This contrasts with the relatively higher levels in the controls (35%) and the schizo-affectives (10%) at this time. Third, the manic-depressives showed some adaptation to the fast. Peak glucose levels occurred late (2 h) and levels at this time were higher than in the pre-fast or refeed tests. The response, however, was comparatively moderate as well as delayed.

The pre-fast and refeed glucose tolerance curves of the schizo-affectives and manic-depressives were somewhat elevated compared to controls. This is consistent with the reports of a higher incidence of diabetic-like responses among manic-depressives⁵ and in patients subjected to long term phenothiazine treatment¹¹, which was the case with the schizo-affectives. Nonetheless, the schizo-affectives reacted

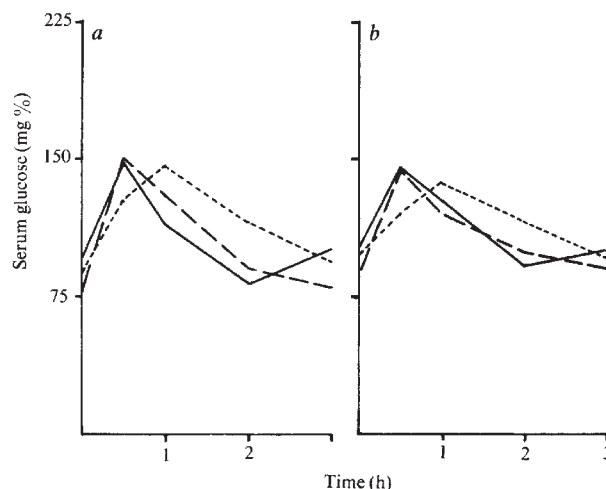
**Fig. 2** Effect of lithium on the glucose tolerance curve of a manic-depressive, patient LD (see text and Table 2). —, Prefast; ---, fast; ···, refeed. a, Without lithium; b, with lithium.

Table 4 Statistical Analysis* of Effects of Fasting on Responses to a Glucose Load

Time (h)	Control Serum glucose (mg% $\pm$ s.e.m.)	P†	Manic-depressive Serum glucose (mg% $\pm$ s.e.m.)	P‡	Schizo-affective Serum glucose (mg% $\pm$ s.e.m.)	P§
Pre-fast						
0	79 $\pm$ 2.80	N.S.	89 $\pm$ 6.55	N.S.	80 $\pm$ 4.43	N.S.
0.5	109 $\pm$ 7.51	N.S.	134 $\pm$ 8.89	N.S.	149 $\pm$ 7.82	<0.01
1	87 $\pm$ 8.59	<0.05	131 $\pm$ 14.43	N.S.	131 $\pm$ 13.97	<0.05
2	65 $\pm$ 5.86	N.S.	80 $\pm$ 3.98	N.S.	93 $\pm$ 11.73	N.S.
3	69 $\pm$ 3.87	N.S.	76 $\pm$ 8.49	N.S.	71 $\pm$ 4.29	N.S.
Fast						
0	76 $\pm$ 4.69	N.S.	66 $\pm$ 3.29	N.S.	69 $\pm$ 6.34	N.S.
0.5	149 $\pm$ 13.27	<0.05	111 $\pm$ 4.71	<0.001	162 $\pm$ 5.51	N.S.
1	206 $\pm$ 7.50	<0.001	137 $\pm$ 7.81	<0.001	216 $\pm$ 11.79	N.S.
2	164 $\pm$ 7.88	<0.05	135 $\pm$ 8.28	<0.01	184 $\pm$ 12.01	N.S.
3	120 $\pm$ 5.39	<0.05	97 $\pm$ 7.16	<0.01	133 $\pm$ 6.91	N.S.
Refeed						
0	88 $\pm$ 4.17	N.S.	79 $\pm$ 3.61	N.S.	80 $\pm$ 6.22	N.S.
0.5	113 $\pm$ 9.93	<0.05	140 $\pm$ 4.71	N.S.	138 $\pm$ 6.66	N.S.
1	85 $\pm$ 2.15	<0.01	127 $\pm$ 12.91	N.S.	113 $\pm$ 7.16	<0.01
2	76 $\pm$ 5.41	N.S.	91 $\pm$ 4.57	N.S.	86 $\pm$ 7.37	N.S.
3	71 $\pm$ 4.13	N.S.	79 $\pm$ 3.14	N.S.	65 $\pm$ 6.49	N.S.

* All results are expressed as mean and standard error of the mean. A two-tailed Student's *t* test was used for analysis in all cases.

† Manic-depressives compared to controls.

‡ Manic-depressives compared to schizo-affectives.

§ Schizo-affectives compared to controls.

to the fast like the controls.

Though we have not yet found a sufficient number of non-medicated volunteers to establish this with certainty, lithium does not seem to be a critical variable in these experiments. The response of the two lithium groups to the fast was different in spite of the similarity between their pre-fast and refeed glucose tolerance curves. The lithium control group responded to the fast like the non-lithium controls. LD's curves were virtually identical with or without lithium.

In further investigations of the glucose metabolism of the manic-depressive, we have noted that the serum insulin levels do not always correlate with the response to a glucose load. While these preliminary data are consistent with a change in overall hormonal balance rather than a change in any single hormone, the complexity of the neuroendocrine system presents problems in the identification of the critical difficulty. Hormonally-mediated responses involve synergistic and antagonistic effects on rate-limiting cellular processes¹², feedback regulation of hormone levels¹³, influences on gene expression¹⁴ and protein synthesis¹⁵ and a critical role of the central nervous system in the organisation of these events¹⁶. Glucose metabolism itself is influenced by insulin, glucagon, catecholamines, glucocorticoids, thyroid and growth hormone¹⁷.

Further, Britten and Davidson¹⁸ have suggested that the expression of mammalian regulatory genes¹⁹ is under hormonal control. A defective regulatory gene could account for the unusual response of the manic-depressive to a fast and still permit successful and appropriate responses to the many stimuli which continuously provoke changes in hormone balance. Since long-lasting modifications in gene expression can be environmentally induced^{20,21}, such a defect in function does not necessarily imply a mutation.

Whatever the origin of the unusual response to the fast, it appears that the manic-depressive is at risk biologically, a view which finds support in the report of the premonitory changes in noradrenaline metabolism in manic attacks²² and the failure to discover immediate precipitating psychological causes for the sudden appearance of a disordered affect²³. It is also apparent that susceptibility to severe affective episodes occurs in individuals with very different biochemical characteristics, a fact which may be of value in diagnosis. Whether the hormonal disturbances associated with a disordered affect in manic-depressives and schizo-affectives are

identical or whether differences occur here also is now a subject of investigation in this laboratory.

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book reviews

Newton's *Principia Mathematica*

Philosophiae Naturalis Principia Mathematica. By Isaac Newton. Third edition with variant readings. Edited by Alexandre Koyré and I. Bernard Cohen, with Ann Whitman. Vol. 1 pp. 547; vol. 2 pp. 368 (Cambridge University: London, August 1972.) £25.

Introduction to Newton's Principia. By I. Bernard Cohen. Pp. 380. (Cambridge University: London, October 1971.) £13.

NEWTON'S *hypotheses non fingo* is almost as well known as his experiments with the prism or his observation of the falling apple. But its meaning is not so clear. Not that the eighteenth and nineteenth centuries were in much doubt about it. In English it meant "I frame no hypotheses" in the words of Andrew Motte's English translation of the third edition of the *Principia*, and in French it meant the slightly different "J'imagine point d'hypotheses" in the words of Voltaire's friend Emilie du Châtelet's posthumous French translation. For almost everyone—especially scientists—it meant facts are safe, hypotheses dangerous, hold to the former, avoid the latter and, in so far as you do that, you will be following the immortal Newton's advice and the success—if not the importance—of your enterprise will be guaranteed.

Newton's practice, however, did not always square too well with this advice. Admittedly, there are no very obvious hypotheses in his first optical paper of 1672, that supreme example of the art of drawing deductions from experiments. But what of the paper of 1675, "An hypothesis explaining the properties of light . . .", with its fantastical explanation of reflection and refraction in terms of an hypothetical ether whose pressure was greater in air than in solid matter, and to which was later joined an *ad hoc* hypothetical "skin" at the inter-surface between air and solid, not to speak of "easy fits" of transmission and reflection to explain partial reflection and the colours of soap bubbles and of Newton's "rings". Newton was evidently prepared to "entertain" hypotheses, even if he did not "frame" them, and even if he only entertained them for the sake of certain great "virtuosos" whose heads ran "much upon hypotheses".

But what of the *Principia* itself, that

arc of the scientific covenant? Even if we suspend judgment on the charge of an hypothetical element in concepts such as force, mass or gravitational attraction with their inextricable mixture of the actual and the ideal, it is impossible to deny the existence of no less than eight named hypotheses at the beginning of Book 3 of the first edition. But in this edition the *hypotheses non fingo* is absent; it appears first in the famous concluding *Scholium Generale* of the second edition. In that edition the number of named hypotheses is reduced from eight to one. In other words, a comparison between the first and second editions of the *Principia* indicates a considerable hardening in Newton's attitude to hypotheses. So that whatever he actually meant by *hypotheses non fingo* in 1713 it is not likely to have been exactly the same as what he would have meant by the same phrase in 1687.

This discovery, made independently by I. B. Cohen and the late Alexandre Koyré, had two important effects: it led to a fundamental reappraisal of Newton's attitude to hypotheses, and to the suggestion by Koyré—since generally accepted—that what Newton really meant by *hypotheses non fingo* was not "I do not frame hypotheses", but "I do not feign hypotheses"—in the manner of Descartes, for example. In the second place, this discovery led to the realisation that Newton's thought did not suddenly become static after the publication of the *Principia* in 1687, and that the various editions of the *Principia* contained a great deal of evidence—hitherto largely ignored—on his changing attitudes to certain key concepts including that of hypotheses. Like some of the best discoveries this one is inclined to seem obvious in retrospect. But it fired Koyré and Cohen to undertake an enterprise of heroic proportions, a variorum edition of the *Principia*.

Of the variorum edition itself I do not propose to say much beyond praising its admirable plan, its beautiful printing and layout, and its excellent critical apparatus. The edition is based on a total of eight texts, those of the three editions, the printers' copy of the first edition, and the four interleaved copies—two each for the first and second editions—kept by Newton himself for

the noting of errors and improvements. The level of accuracy attained in such a work is evidently of cardinal importance. This is something which can only be determined by use over a long period. I have no doubt it will be found to measure up to the most exacting standards.

The accompanying introduction by I. Bernard Cohen is mostly taken up with the history of the preparation of, and reaction to, the three editions of the *Principia*. Of this history a great part is devoted to the story of the long and gradual process of correction and emendation by which the first edition of 1687 was transformed into the greatly improved second edition of 1713 from which the third edition of 1726 differed little. Apart from Newton himself, pursuing the endless task of correction with unswerving and unhurried determination, there appear a number of other figures. Halley, who had played such a large part both in the composition and publication of the first edition, and who wrote the first review of it. The Italian Fatio De Duiller to whom Newton seems to have been powerfully attracted for a time before his scientific judgment reasserted itself, and whose notes on the *Principia* are interesting not for their intrinsic merit, but for what they tell us of the early stages in the preparation of the second edition. David Gregory, who made a more intensive study of the *Principia* than anyone apart from Halley and Newton himself, but who was disappointed in his plan to publish a commentary, or possibly even in becoming editor of the second edition. Richard Bentley, under whose masterful hand the printing of the second edition was actually begun in 1708, only to be discontinued after eight pages, probably because Newton suddenly realised that Bentley's mathematical inadequacies would have necessitated his checking all the proofs himself.

Finally, the somewhat tragic figure of Roger Cotes, Plumian professor of mathematics at Cambridge, who had the necessary mathematical ability, the tact not to offend Newton, and the strength of character to stand firm against him when he knew he was in the right and Newton in the wrong. As the Newton-Cotes correspondence shows, Newton's debt to Cotes was enormous as regards his overseeing of the printing, his discovery of errors,

suggestions for improvements, and provision of the famous preface with its brilliant exposition of the Newtonian philosophy of science including Newton's very subtle attitude to gravitational attraction. Sad to say, as Professor Cohen admits, there is no trace in the Newton-Cotes correspondence of any word of thanks from Newton to Cotes for all his enormous labours: and Newton's famous epitaph to Cotes following his early death in 1716: "If Mr Cotes had lived we might have known something" seems to imply that Newton never fully appreciated Cotes' contribution, since but for Cotes the second edition of the *Principia*, if it had ever appeared, would certainly have been a much less important edition than it actually was.

Throughout almost the whole of the introduction Cohen shows an easy mastery of a vast mass of source material, follows the good example of Newton himself in the matter of English prose style, and strikes a happy balance between an uncritical reproduction of every scrap of relevant information and a too shallow and superficial treatment. Only in chapter 3—"Steps toward the *Principia*"—does he seem to falter a little. One reason for this may have been uncertainty what to say here about the technical, dynamical aspects of the early steps in the composition of the *Principia*, about which an additional volume of commentary is promised. In fact the treatment given is not only confused on occasion, especially in the discussion of the various versions of the tract *de Motu*, but it also contains a number of somewhat hasty judgments which will hardly command universal assent.

Thus it seems to me somewhat improbable that in 1684 Newton was admired by the *cognoscenti* not only for his discoveries in mathematics but also for his "grasp of the fundamentals of dynamics" (page 47); in the first place Newton was very secretive about his early work in the subject, and in the second place "the fundamentals of dynamics" had, in part, still to be formulated. Equally suspect is the assertion (page 52) that Newton "apparently learnt of Kepler's law of areas in 1678 and at once was able to solve the problem of Keplerian planetary motion" (my italics). This view, originally put forward by D. T. Whiteside¹, was very improbable *a priori*, and was then effectively ruled out by J. L. Russell's² demonstration that Kepler's laws had been much more familiar to astronomers in the first half of the seventeenth century than had previously been thought to be the case—Russell lists four authors, Herigone, Riccioli, Boulle and Wallis, who all cited Kepler's second law in its exact, areal form before 1660. Admittedly, the second law

was evidently the least familiar, and its dynamical significance remained entirely hidden before 1679. But it is stretching credulity to suppose that Newton had not noticed—and deeply pondered on—this extraordinary law before 1679. Curious, too, is the assertion (page 53) that "only near or at the surface of the Earth did he (Galileo) conceive of a true linear inertial motion" or the truly extraordinary remarks (page 61) that the tract *de Motu* "does not yet bear the mark of genius" and contains "no hint of the grandeur of the *Principia* to come": after all, it was in this tract that Newton first solved the problem of Kepler motion, the very cornerstone of the whole *Principia*.

Critics will inevitably differ, as critics do, on these and other minutiae. All will be agreed, however, on the magnitude of the achievement of the variorum edition and its introductory volume. Barring the unlikely appearance of important new Newton manuscripts, the whole work will surely remain definitive well into the next century, if not beyond. Alexandre Koyré, I. B. Cohen and their band of devoted assistants have deserved well of the republic of letters, and I can only regret that Koyré did not live to see the completion of the work originally conceived by him and Cohen in 1956. J. W. HERIVEL

¹ Whiteside, D. T., *Br. J. Hist. Sci.*, 2, 117-137 (1964).

² Russell, J. L., *Br. J. Hist. Sci.*, 2, 1-24 (1964).

Rockets and roubles

The Origins and International Economics of Space Exploration. By Bernard Lovell. Pp. viii+104. (Edinburgh University: Edinburgh, 1973.) £1.50.

It is excellent to find such a wealth of information in so slim a volume. I especially enjoyed the succinct and clear historical part (the first quarter of the book). To describe the early military history of the rocket, to disentangle the labours of Tsiolkowsky, Goddard and Oberth, to demonstrate the independence of Soviet work from the earlier German efforts, to explain the administrative tangles in the United States before NASA got going, and all in some twenty very readable pages is most valuable. Much of the rest of the book gives data on costs and on numbers of launchings, and examines the relative sizes of civil and military space efforts in both United States and Soviet Union all in very convenient and easily assimilable form, and describes briefly, and perhaps a bit conventionally, the various benefits of space activity. Science in particular is dealt with too briefly for anybody to learn much from it, but more justice is

done to the applications. The contrast of the cost of manned and unmanned space activities is well brought out, and a somewhat implicit admiration, richly deserved, for the political vision, superb decision taking, and technological brilliance of the Apollo programme is understandably followed by a much more dubious attitude to later manned activities (Skylab and Shuttle) to which so much highly promising space science (high energy astronomy and planetary observation) is being sacrificed.

However much I admire these concise descriptions, I am distinctly less thrilled by Sir Bernard Lovell's more philosophical comments, comments that are far too brief and therefore lack clarity and depth. If there was not the room to develop the argument more fully (which might well have been fascinating), then the commentary should have been left out. As it is, the reader is simply confused and irritated by the sputtering that first points one way, then the other, never addressing itself to any problem for long enough, never resolving the contradictions.

To give some examples: after soberly describing the V1 and V2 campaigns, and the casualties caused, he rightly says that these weapons could have affected the war far more but for the bombing of Peenemünde, only to be "filled with dismay and an unresolved conflict" by the thought that this bombing was made possible by the blind bombing aid which he had helped to develop "illuminating for destruction the genesis of one of the greatest scientific developments of all time . . .". His dismay is hard to understand, given the facts of the situation. Similarly, on page 86 he stresses that the United Kingdom's space expenditure was so minute that, if transferred to education, health and social security, it would have hardly made any difference, but on page 87 he speaks of wasting "these extremely large sums" (that is, those applied to space, perhaps including this time the other European countries). Whether depending on the United States for launching rockets (page 87) is the bad thing it is implied to be at least deserves to be debated. I, too, am keen on space, but would find it hard to sustain (though I would enjoy reading a good argument on the issue) the assertion that "many of the urgent problems associated with the predicted doubling of the world's population can be alleviated by space applications" (page 88). These are important and controversial issues on which reasoned argument would have been most welcome, but little benefit is derived from over-brief and often unclear assertions.

But my gripe about what is not in the book should not cloud my advice to all interested in space to read it for its factual content. H. BONDI

Food and development

The Nutrition Factor: A Study jointly by the Foundation for Child Development and the Brookings Institution. By Alan Berg. Portions with Robert J. Muscat. Pp. xii+299. (Brookings Institution: Washington DC; Allen and Unwin: London, August 1973.) £4.50 boards; £1.75 paper.

THIS is a worthwhile book. It is concerned with the improvement of nutritional health in the developing countries but differs from most other books on the subject in that it goes to great length to point out that such improvements have an important role in national development. This is not a practical manual in that it does not set out step-by-step instructions—the approach is not so dogmatic; rather it deals in a philosophical way with the background to the problem and suggests principles for effecting change. The book will surely become essential reading for students of applied nutrition and for those administrators and civil servants in the developing countries who have need to convince their rulers that the malnourished members of the community are not economically insignificant.

The starting point of Dr Berg's philosophy is best summed up in his own words: "malnutrition adversely affects mental development, physical development, productivity, the span of working years—all of which significantly influence the economic potential of man". It would be easy to quibble with the opening chapters in which Dr Berg justifies this statement. Perhaps too little theoretical proof is available but few scientists or doctors would doubt its basic truth, and the aim of the book is to encourage national action rather than stimulate further fundamental research.

A whole chapter is devoted to the cynical but often encountered argument that improved nutrition will merely exacerbate the population problem. The nutritional adviser will need to combat this line of attack; the chapter provides some valuable ammunition. People in developing countries will not readily accept contraception until they can be sure that their children, in particular sons, will survive.

Dr Berg quite rightly believes that significant nutritional improvements at a community level will materialise only if the problem is viewed from a national perspective and tackled by broadly based preventive measures. He points out the limitations of the "person to person diagnostic and curative approach"; however essential this may be to the ministries of health, national planners need to adopt a more comprehensive policy. The main possibilities discussed are: economic growth leading

to higher incomes and hence better nutrition, improved agricultural production and farming technology, nutritional education, the formulation of new foods and nutrient fortification, the encouragement of new food industries and, finally, feeding children through nationally organised public programmes. Each topic is dealt with and commented on in an instructive manner. The conclusion is that each approach has its valuable features but none can achieve the desired goal alone; an integrated plan is essential.

The main stimulus for Dr Berg's approach to malnutrition has been his experience of India, and the narrative ends with a discussion of the lessons learnt there both from the general situation and from the crisis brought about by the Bihar famine. Dr Berg considers that the beginnings of a national programme in India can be looked upon as a prototype by other nations.

The book concludes with numerous tables and appendices providing valuable facts and figures together with a comprehensive bibliography.

This monograph purposefully confines itself to the food and dietary

factors involved in malnutrition and readers might, if they did not read carefully the penultimate paragraph of the first chapter, be led to believe that these were the only ones that matter. Perhaps it is of value to dispel this idea by again quoting Dr Berg: "In a systematically planned search for remedies to nutritional problems, many non-standard nutritional measures will be considered. Protected water supplies, waste disposal and other forms of environmental sanitation, immunisation against certain diseases, for instance, all relate to the incidence of malnutrition, its relative severity and the reduction of mortality. Although such measures are not normally thought of as nutritional measures, they may be significant determinants of nutritional status, or, more likely, essential in combination with the food-related measures that this study focuses on".

These words emphasise still further the enormity of the problem facing those who are trying to improve nutritional health in the developing world. Little wonder Dr Berg advocates such far-reaching preventive measures; his book is timely and warmly welcomed.

R. G. WHITEHEAD

Solid state electrochemistry

Electrochemistry of Metals and Semiconductors: The Application of Solid State Science to Electrochemical Phenomena. By Ashok K. Vijh. Pp. xiv+297. (Dekker: New York, May 1973.) \$23.50.

THE electrode-solution interface has been studied from the viewpoint of solid state physics by many workers since the pioneer paper of Grimley and Mott in the 1947 Faraday Discussion. During these years the theory of redox reactions at metal or semiconductor electrodes has been developed by Gerischer on the basis of the classical hydrogen overvoltage paper of R. W. Gurney; a good many practical applications have been examined, and some established in industry. Dr Vijh works in the Hydro-Quebec Institute of Research, and has published a number of papers in recent years. His book sets out to provide first a rudimentary introduction to the solid state (really band theory) to electrochemists unfamiliar with this topic. There follow eight chapters, including three on electrode reactions (general principles, elemental semiconductors, bulk compound semiconductors), then anodic oxidation of metals and non metals, and the effect of surface films on electrode reactions. In chapter 7 the solid state properties of metals are related to their electrochemical behaviour, and in chapter 8 electrochemistry in the solid state (as solid electrolytes and proton

conduction in ice, and so on) is studied. A final chapter deals with industrial aspects, some ten applications being noted from fuel cells and batteries to mineral processing.

Most of my criticisms turn on points of detail, since the book as a whole usefully fills an important gap in the monographic literature. To say that semiconductors show a non-Ohmic behaviour under ordinary conditions (p. 21) is surely rather misleading. In view of its importance more attention should have been given to explaining the Fermi level, and its relation to energy bands and impurity levels. The printing of the various Galvani potentials on p. 191 *et seq.* leaves much to be desired. On p. 21 intrinsic conductivity is rightly written as $\sigma_0 \exp(-E_g/2kT)$, on p. 131 wrongly as $\sigma_0 \exp(-E_g/kT)$. There is published evidence for the free energy of activation for oxide growth proportional to the oxide thickness (p. 132) but this is not quoted.

Putting such detailed matters on one side, the book is easy to read, and should perform a valuable function in introducing research workers to an important new field of interfacial activity, and we may expect it to stimulate further efforts in this direction. Biological systems are rightly excluded from the present volume, but there are also signs of a growing activity in this area.

D. D. ELEY

Animal chromosomes

Animal Cytology and Evolution. By M. J. D. White. Third edition. Pp. viii+961. (Cambridge University: London, June 1973.) £19; \$55.

WRITERS of general accounts of chromosomes before Professor White used to attempt to cover both plants and animals. This policy was justified by the uniformity in principle and also by the complementarity in detail of the two kingdoms: the chromosomes of plants were more useful in showing the causes of evolution; those of animals revealed above all its consequences. Or, to put it in another way, the immense range of experiments, chemical and physical, genetic and physiological, that have been successful with plant chromosomes provide quite a different picture from the descriptive variety which is revealed by a systematic survey of animal chromosomes.

Professor White was the first, in successive editions of this book, to try to disentangle the animals from the plants, the consequences from the causes. He took this course, no doubt, because the animals suited him extremely well. He is the most experienced specialist in animal cytology in the world and he excels in describing and illustrating the wealth of variation of chromosomes in their form and behaviour as they operate the multifarious genetic and developmental systems of animals.

Naturally Professor White has to refer to the principles of chromosome behaviour revealed by plants, but he does so reluctantly and piecemeal, lightly suspending his judgment whenever the issue becomes serious. Evidently he does not recognise the contrast or the complementarity between the opposed evolutionary aspects of chromosome study, and no one from reading this book could pick up any such disturbing notions. There are many students of chromosomes, to be sure, who will not want to be disturbed, and for them Professor White has provided a solid and satisfactory work of reference.

C. D. DARLINGTON

Scottish shores

The Coastline of Scotland. By J. A. Steers. Pp. xx+335 (64 plates). (Cambridge University: London, 1973.) £10.50; \$31.50.

It is now almost thirty years since Professor Steers's monumental work on the coastline of England and Wales appeared. The natural sequel was a similar study of the coasts of Scotland and now, in retirement, he has, with characteristic thoroughness, set out his views on the form and origin of some

of the finest coastal scenery in Britain. The book is cast in much the same mould as his earlier work with a few introductory chapters covering general themes to be followed by a survey of the whole coastline, including all the offshore islands. Inevitably he has drawn heavily on the work of others, for although he has walked over nearly all the coastline he describes, detailed studies of individual sections proved impossible.

Fortunately the geological memoirs of Scotland are particularly detailed as regards coastal sections and many of the early volumes, like that of Caithness, contain a wealth of physiographic information. There is, however, a general lack of research papers similar to those found in the local journals in England. In part this deficiency has been made good by the research efforts of Scottish geographers, particularly those based at Aberdeen. Professor Steers has used their studies to advantage in his text to provide local colour for what is basically a factual account.

Undoubtedly the book will prove of great value to the specialist but seems hardly likely to make easy reading for the amateur enthusiast with an interest in our coastal setting. It is to be regretted that Professor Steers has not felt able to draw upon his vast experience to be more speculative, not only posing problems but giving a lead as to their solution. For the island of Ailsa Craig, for example, he contents himself with a factual account of its geology and vegetation (pages 103-4) without real reference to the interestingness feature which dominates its eastern coastline. Again in the entrance to Inverness Firth he makes detailed reference to the classic work of Ogilvie, carried out over fifty years ago, when the relative roles of tidal currents and wave action were appreciated for the first time. Since then much new evidence has been forthcoming on this important aspect of coastal evolution, particularly in relation to sediment transport by residual water movements. This is a theme which Professor Steers could have properly developed without recourse to detailed research, for the evidence is there in the bottom configuration of the firth.

The book is well illustrated with maps. It has a centre spread of excellent photographs from the Cambridge University collection of air views, although as they do not occupy the full page size they give the book an old-fashioned appearance. Inevitably a comparison will be made with his earlier book on the coastline of England and Wales. It is a sad fact that as book prices have risen steeply in the last few years the quality of production has declined.

A. H. W. ROBINSON

Sea urchin development

Development Biology of the Sea Urchin Embryo. By Giovanni Giudice. Pp. x+469. (Academic (Harcourt Brace Jovanovich): New York and London, August 1973.) \$32.

THERE is a great need for more sympathetic communication between experimental embryologists and molecular biologists; and this book, by drawing together the enormous amount of information on the morphogenesis, ultrastructure, biochemistry and molecular biology of the sea urchin embryo, makes a timely and scholarly contribution towards this end.

For the reader with no formal background in embryology there is a lucid account of the experiments of the early 1900s that still provide the basis of current ideas about the programming of sea urchin development; for example, the proposed existence of gradients of potentiality to form ectodermal or endodermal structures emanating from the 'animal' and 'vegetal' poles of the egg respectively. There is fascinating reading, too, in the descriptions of sperm penetration, the behaviour of the male and female pronuclei, and the membrane changes following fertilisation—examples of phenomena which, as yet, have no molecular explanation.

The reasonably up to date summary of research on the sea urchin embryo from a more biochemical viewpoint contains a clear description of experiments which led to such ideas as the existence of 'masked' maternal messenger RNA, and the synthesis of large amounts of histone and histone messenger RNA by the early blastula. But this is a rapidly moving field and it is a pity that the book was finished (early 1972) before the application to the sea urchin embryo of important new advances in the technology of isolating and translating messenger RNAs (see, for example, *Nature*, **245**, 8; 1973) could be included.

Dr Giudice's book is also a useful source of practical information about handling embryos, separating cells, problems of permeability, and so on. It is clearly written, with paragraphs summarising the main problems to be solved and the information available. Beyond this synthesis, however, the author makes no attempts to provide new insights or theories. Finally, the reader is left in no doubt as to the disadvantages, as well as the advantages, of the sea urchin in studying problems of developmental biology, for Dr Giudice is no slavish devotee of his research material. The main disadvantage is, of course, that no genetic analysis of development can be made because it is very difficult to rear the embryos in the laboratory beyond the pluteus stage.

BRIGID HOGAN

matters arising

Douc Langurs

SIR,—Relevant to Dr Kavanagh's observations (*Nature*, 239, 406; 1972) of food sharing among captive douc langurs (*Pygathrix nemaeus*), I should like to describe some of my own observations of these monkeys in the wild. Between May 1967 and May 1968, I made fourteen visits to Mount Son Tra (elevation 696 m above sea level), about 10 km north of Da Nang on the northeast coast of South Viet Nam. Here I watched two groups of douc langurs as they fed in the canopies of the trees which they inhabited. At the time the animals were relatively tame, benefiting from partial protection afforded by being on a military base, but recently they have declined on Son Tra (Van Peenan *et al.*, *Mammalia*, 35, 127; 1971). The first groups consisted of four adults, apparently one male and three females (distinguishable when external genitalia were visible), and a baby. The second group consisted of five adults (apparently two males and three females), a half-grown monkey and a baby. The members of a group foraged in close

proximity, usually within 10 m of each other, although not always in the same tree, and they rarely came to the ground. Often two or three animals fed in the same terminal clump of foliage, and occasionally when one pulled down a branch, an adjacent individual also fed on it. This type of passive food sharing was noted by Kavanagh.

I also observed active food sharing on two occasions. In the first group of langurs an adult female bit a small section of leafy twig and passed it to the male who quickly stripped off and ate the leaves. The two animals continued to forage within 2 m of each other, but no further interaction was noted for about 30 min. Then, however, the female solicited copulation from the male who subsequently mounted her, but the animals were disturbed by a passing vehicle before intromission was achieved, and they disappeared from view. In the second group I saw a similar case of food being passed from an individual of unknown sex to a female who had a baby clinging to her. I observed no further inter-

action between these two animals in the few minutes before they disappeared in the foliage.

In each case the passing of food seemed to be spontaneous. The recipient did not appear to threaten or beg, although as Kavanagh pointed out there may be subtle communication patterns inapparent to the human observer. Further observations of these animals in the wild may reveal, as Kavanagh suspected, that food sharing is not rare in family groups in natural conditions. I should add that the species is endemic to the Indochina Region, including Hainan Island, where much of its environment has suffered profound changes, and it is considered an endangered species¹.

Yours faithfully,

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¹ *Red Data Book: Mammalia* (IUCN, Morges, 1972).

obituary

Frederick James Dent

FREDERICK JAMES DENT, FRS, OBE, leader of the 1971 MacRoberts Award winning team, died on October 5 at his home in Malta after a short illness. He was 67.

Dr Dent was born in 1905 and was educated at the Modern School, Leeds. He was a student at the University of Leeds under Professor J. W. Cobb, CBE, obtaining his doctorate in 1929 for an original piece of work on the carbon steam reaction. In the same year he was appointed research chemist in charge of gas production investigations carried out by the Joint Research Committee of the Institution of Gas Engineers and the University of Leeds. In this period he conducted a classical study of the water gas process on the full scale which led to a detailed laboratory investigation of

the gasification of coal in steam and oxygen at high pressure.

It was during this latter work in 1937 that he discovered that methane produced during gasification under pressure was formed by the direct action of hydrogen on the coal substance. During this same period he carried out a study of the catalytic synthesis of methane from carbon monoxide and hydrogen and identified and solved the problems associated with the application of this reaction to the production of town gas. Both these discoveries, the one relating to coal hydrogenation and the other to methanation, have currently enormous significance in the work going on today in the United States to counter the growing natural gas shortage by the gasification of coal to methane as a substitute for natural gas.

On the day of his funeral the success-

ful start-up of a methanation demonstration plant at the coal gasification plant of Scottish Gas, at Westfield, was announced in the press. The previous week this plant, the essential features of which are directly based on Dent's work, produced the first substitute natural gas from coal on a commercial scale, fore-running new plants in the United States which will produce billions of cubic feet of synthetic natural gas per day in the future. The new generation of processes now being developed in the United States for the production of substitute natural gas will practically all also incorporate the coal hydrogenation reaction which he discovered at Leeds and first demonstrated on a pilot plant scale at the Poole Research Station of the Gas Research Board.

It was at Poole, where he was Assistant Director of the Gas Research Board

from 1943–51, that many of Dent's studies on gas production processes, both paper and experimental, were initiated. For example, it was here that the first coal and oil were hydrogenated in fluidised beds and methanol was first catalytically gasified. It was not until he was appointed Director of the Gas Council's Midlands Research Station in 1951, however, that the funds needed to enable him to develop his ideas to commercial reality became available.

The move to Birmingham and later to Solihull coincided with the start of a change in the fuel situation and a shift in emphasis from coal to oil as a feed-stock for gas production. The work on the hydrogenation of oil to gaseous hydrocarbon—rich gas accelerated with the construction of a large fluidised bed hydrogenation pilot plant, followed later by two more specially designed ones which led to the development of the Gas Recycle Hydrogenerator. This process, designed specifically to hydrogenate distillate oils, was quickly adopted by the industry and more than fifty units were installed in this country and abroad for the enrichment of over one billion (10⁹) cubic feet per day of town gas.

At the same time, he pursued the development of the Catalytic Rich Gas (CRG) process and, with a highly effective catalyst, brought this also successfully to the commercial stage. Some forty-four units of CRG-based town gas plants have been built, ten of them abroad, while more recently two streams of CRG-based substitute natural gas plants have been commissioned in the United States with a further nine streams in various stages of construction. A further plant is shortly to start up in Japan. The combined thermal output of all these plants, some of which are the biggest in the world, matches the size of the present British gas industry.

During this period of intense activity on oil processes, Dent found time to extend the development of the Lurgi fixed bed high pressure coal gasifier to slagging operation. Two pilot plants were built and operated in the late 1950s and early 1960s and the world's first high pressure steam and oxygen slagging gasifier was satisfactorily developed and demonstrated at Solihull to prove the potential advantages which Dent had predicted for this system from his earlier laboratory studies at Leeds. Increasing interest is currently being shown in this gasifier by companies and government agencies concerned with coal gasification in the United States.

Dent's outstanding contributions to the development of processes for the gasification of coal and oil received worldwide acclaim. His work was recognised by the award of the Birmingham Medal (the highest award of the Institution of Gas Engineers) the Melchett Medal of the Institute of

Fuel, the Walton Clark Medal of the Franklin Institute of Philadelphia and the Coal Science Medal of the British Coal Utilisation Research Association. He was awarded an OBE in 1958 and was elected a Fellow of the Royal Society in 1967. These honours culminated in 1971 with the engineering profession's highest prize, the MacRobert Award, which he received jointly with the chief members of his team.

His success stemmed perhaps mostly from his ability to interpret his creative scientific work into practical chemical engineering and process technology. His unbounded enthusiasm and the real enjoyment he had in his work, however, produced the drive in others which was essential to the rate of progress he achieved, a factor which he always generously recognised. He was modest to a fault, impatient with the less well informed and demanded complete technical integrity. Amongst those with a common interest in gasification and gas production processes he made many friends who will retain a memory of a cheerful and kindly man with a deep and detailed understanding of both the theoretical and practical aspects of his subject.

He leaves a widow, a son, a daughter and three grandsons.

Announcements

Appointments

Professor A. D. Bradshaw is to be chairman of the **Nature Conservancy Council's** advisory committee on science.

Derek A. Ratcliffe has been appointed Chief Scientist of the **Nature Conservancy** and **Ian Prestt** is to be Deputy Director.

Professor W. A. Holmes-Walker has been appointed Director of the **British Plastics Federation**.

Harry Hookway, Chief Executive and Deputy Chairman of the **British Library** has been elected President of the Institute of Information Scientists.

Joan Eileen Walsh has been appointed to be Professor of Numerical Analysis of the **University of Manchester**.

Miscellaneous

The **Lomonosov Gold Medals** for 1973 have been awarded to **Academician Aleksandr Pavlovich Vinogradov** for his work in geochemistry and to **Vladimir Zoubek** for his work in geology.

The **Council of Systematics Association** Department of Biology plan to offer an Essay Prize in 1974.

Erratum

IN the article "Pharmacologically induced behavioural supersensitivity to apomorphine" by Daniel Tarsy and Ross J. Baldessarini (*Nature new Biol.*, 245, 262; 1973) the following sentence should be added. "Dr Tarsy is a member of the Department of Neurology, Boston University School of Medicine and the Boston Veterans Administration Hospital, Boston, Massachusetts".

Reports and Publications

not included in the *Monthly Books Supplement*

Great Britain and Ireland

A Guide to the Use of Terms in Plant Pathology. Prepared by The Terminology Sub-Committee of the Federation of British Plant Pathologists. (Phytopathological Papers, No. 17.) Pp. iv + 54. (Kew, Surrey: Commonwealth Mycological Institute, 1973.) £1.30. [2611]

Principles of Free Radical Chemistry. By Dr J. I. G. Cadogan. (The Chemical Society Monographs for Teachers, No. 24.) Pp. 83. (London: The Chemical Society, 1973.) £1. [2811]

The British Council. Annual Report, 1972/73. Pp. 102. (London: The British Council, 1973.) [2811]

The Royal Society. Report of Council for the year ended 31 August, 1973. Pp. 109. (London: The Royal Society, 1973.) [2911]

Research Supported by the Social Science Research Council 1973. Pp. 318. (London: Social Science Research Council, State House, High Holborn, 1973.) 75p net. [2911]

Inter-Research Council Committee on Pollution Research. Cadmium in the Environment. (Report of a Seminar held at Alhambra House, Charing Cross Road on 15 March, 1973.) Pp. 39. (London: Natural Environment Research Council, 1973.) [3011]

Other Countries

Tecnia Generale del Lavoro Umano, Volume Primo. By Pier Sergio Vassallo. Pp. 101. (Milano: Pier Sergio Vassallo, c/o CFM-Via Clerici 10, 1973.) [2611]

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Tropical Agronomy, 1972/1973. Pp. 128. (Brisbane: CSIRO, 1973.) [2611]

Dynapol: Forging Safer Links in the Food Chain. Pp. 16. (Palo Alto, California: Dynapol, 1454 Page Mill Road, 1973.) [2611]

Republic of South Africa: Department of Industries. Sea Fisheries Branch Investigational Report No. 101: Growth of the South African Maasbanker *Trachurus trachurus* Linnaeus and Age Composition of the Catches 1950–1971. By N. D. Geldenhuys. Pp. 24. (Sea Point, Cape Town: Sea Fisheries Branch, Beach Road, 1973.) [2711]

Australia: Commonwealth Scientific and Industrial Research Organization. Twenty-fifth Annual Report, 1972/1973. Pp. 99. (Melbourne: CSIRO, 1973.) [2811]

Republic of South Africa: Department of Industries. Division of Sea Fisheries Investigational Report No. 98: Marine Alpha-Radioactivity Off South Africa. 1. Gross Alpha-Activity, Radiation Dose, Alpha-Spectrum and Variations in Alpha-Activity of Marine Life. By L. V. Shannon. Pp. 80. (Sea Point, Cape Town: Division of Sea Fisheries, Beach Road, 1973.) [2811]

Our Agricultural Future. By Dr M. S. Swaminathan. (Sardar Patel Memorial Lectures, 1973, at India International Centre, New Delhi.) Pp. 54. (New Delhi: All India Radio, 1973.) [2811]

Priorities for Action: Final Report of the Carnegie Commission on Higher Education, with Technical Notes and Appendixes. Pp. x + 243. (New York and London: McGraw-Hill Book Company, 1973.) \$4.95. [2811]

Nuclear Energy Agency, OECD. Radiation Protection Standards for Gaseous Tritium Light Devices. Pp. 23. (Paris: Nuclear Energy Agency, OECD, 1973.) [2811]

Republic of Cyprus: Ministry of Agriculture and Natural Resources. Annual Report of the Geological Survey Department for the year 1972. By Y. Hadjistavrinou. Pp. 33. (Nicosia: Geological Survey Department, Ministry of Agriculture and Natural Resources, 1973.) [2811]

OECD. Nuclear Energy Agency. Thirteenth Annual Report of the ORCD Halden Reactor Project 1972. Pp. 178. (Paris: OECD, Nuclear Energy Agency, 1973.) [2811]